CANADIAN JOURNAL OF RESEARCH

VOLUME 28 OCTOBER, 1950 NUMBER 5

— SECTION C —

BOTANICAL SCIENCES

Contents

	Page
Natural Anchoring of the Red Alga, *Gracilaria confervoides* (L.) Grev. on Unstable Bottom by Association with an Annelid Worm—*R. W. Pillsbury*	471
Two New Outbreaks of *Phomopsis lokoyae* in British Columbia—*G. P. Thomas*	477
Three Years of Evapotranspiration at Toronto—*Marie Sanderson*	482
Effect of Pasmo Disease on Seed Yield and Thousand Kernel Weight of Flax—*W. E. Sackston*	493
Storage of Isolated Chloroplasts Without Loss of Photochemical Activity—*P. R. Gorham and K. A. Clendenning*	513
Studies of Canadian Thelephoraceae. VI. The *Peniophora rimicola* Group—*H. S. Jackson*	525
Studies in Racial Trends and Constancy in *Cladosporium fulvum* Cooke—*D. L. Bailey*	535
Studies of Coprophilous Ascomycetes. I. *Gelasinospora*—*Roy F. Cain*	566

NATIONAL RESEARCH COUNCIL
OTTAWA, CANADA

CANADIAN JOURNAL OF RESEARCH

The *Canadian Journal of Research* is issued in six sections, as follows:

A. Physical Sciences	D. Zoological Sciences
B. Chemical Sciences	E. Medical Sciences
C. Botanical Sciences	F. Technology

For the present, Sections A, C, D, and E are to be issued six times annually, and Sections B and F, twelve times annually, each under separate cover, with separate pagination.

The *Canadian Journal of Research* is published by the National Research Council of Canada under authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research. The *Canadian Journal of Research* is edited by a joint Editorial Board consisting of members of the National Research Council of Canada, the Royal Society of Canada, and the Chemical Institute of Canada.

Sections B and F of the *Canadian Journal of Research* have been chosen by the Chemical Institute of Canada as its medium of publication for scientific papers.

EDITORIAL BOARD

Representing National Research Council	*Representing* Royal Society of Canada	
Dr. H. P. Armes (*Chairman*), Dean of the University, University of Manitoba, Winnipeg, Man.	Dr. G. M. Volkoff, Professor of Physics, University of British Columbia, Vancouver, B.C.	Section III
Dr. Otto Maass, Macdonald Professor of Physical Chemistry, McGill University, Montreal, P.Q.	Dr. J. W. T. Spinks, Dean, College of Graduate Studies, University of Saskatchewan, Saskatoon, Sask.	
Dr. J. H. L. Johnstone, Professor of Physics, Dalhousie University, Halifax, N.S.	Dr. H. S. Jackson, Head, Department of Botany, University of Toronto, Toronto, Ont.	Section V
Dr. A. Bertrand, Professor of Bacteriology, Faculty of Medicine, University of Montreal, Montreal, P.Q.	Dr. E. Horne Craigie, Department of Zoology, University of Toronto, Toronto, Ont.	

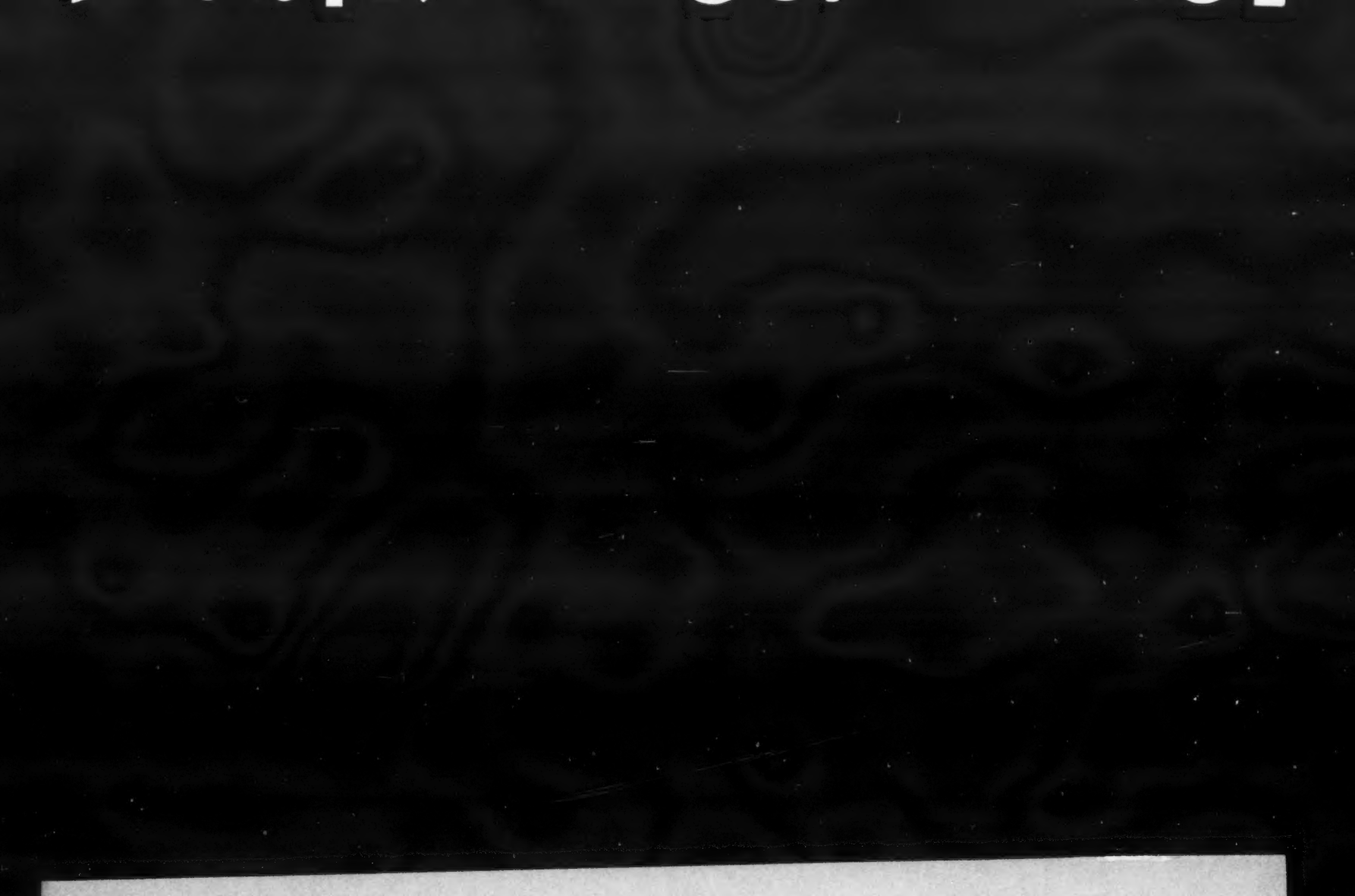
Ex officio	*Representing* The Chemical Institute of Canada
Dr. Léo Marion, Editor-in-Chief, Division of Chemistry, National Research Laboratories, Ottawa, Ont.	Dr. H. G. Thode, Department of Chemistry, McMaster University, Hamilton, Ont.
Dr. H. H. Saunderson, Director, Division of Information Services, National Research Council, Ottawa, Ont.	

Editorial Committee

Editor-in-Chief,	Dr. Léo Marion	Editor, Section D,	Dr. E. Horne Craigie
Editor, Section A,	Dr. G. M. Volkoff	Editor, Section E,	Dr. J. B. Collip
Editor, Section C,	Dr. J. W. T. Spinks Dr. H. G. Thode	Editor, Section F,	Dr. J. A. Anderson Dr. G. M. Volkoff Dr. H. G. Thode
Editor, Section C,	Dr. H. S. Jackson		

Manuscripts should be addressed:

Editor-in-Chief,
Canadian Journal of Research,
National Research Council, Ottawa, Canada



NOTICE

The Canadian Journal of Research is at present published in six sections, A to F. Starting with January 1, 1951, these sections will be published as separate journals under distinctive names and the designation Canadian Journal of Research will no longer be used. The present names and the corresponding new names are as follows:

Present Name	New Name
Canadian Journal of Research, Section A (Physical Sciences)	Canadian Journal of Physics
Canadian Journal of Research, Section B (Chemical Sciences)	Canadian Journal of Chemistry
Canadian Journal of Research, Section C (Botanical Sciences)	Canadian Journal of Botany
Canadian Journal of Research, Section D (Zoological Sciences)	Canadian Journal of Zoology
Canadian Journal of Research, Section E (Medical Sciences)	Canadian Journal of Medical Sciences
Canadian Journal of Research, Section F (Technological Sciences)	Canadian Journal of Technology

In order to preserve continuity the present sequence of volume numbers will be retained, and in each case the volume for 1951 will be Volume 29.

The subscription rates for the Journals will remain as at present.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 28, SEC. C. OCTOBER, 1950 NUMBER 5

NATURAL ANCHORING OF THE RED ALGA, *GRACILARIA CONFERVOIDES* (L.) GREV. ON UNSTABLE BOTTOM BY ASSOCIATION WITH AN ANNELID WORM[1]

BY R. W. PILLSBURY

Abstract

Clumps of *Gracilaria confervoides* (L.) Grev. several meters long and several kilograms in weight have been found growing on clean sand bottom. These plants do not arise from the usual discoid holdfast on a buried stone, etc., but each is anchored by enwrapment of main strands to the tube of a worm, *Diopatra ornata* Moore. This enwrapping was observed to occur by mechanical obstruction of loose hanks of the alga by projecting worm tubes during extreme low tides on winter nights. In three months the alga was cemented to the tube by unexplained action. A coaction of alga and tube worm apparently exists that establishes plant shelter for small animals on an otherwise unsuitable substratum, thus increasing the complexity of the community on such sites. It is suggested that perennating marine algae produce much the same seasonal shelter for animals as do the deciduous plants of terrestrial communities.

Introduction

On the coast of British Columbia the red alga *Gracilaria confervoides* (L.) Grev. occurs on sandy, muddy, or gravelly bottoms in more or less sheltered places. It grows in abundance on certain sandy beaches where there is little or no visible anchorage for rootless plants. Yet there is here, besides some rooted *Zostera*, an abundance of large plants of *Gracilaria* averaging 1.6 m. in length, commonly reaching 3 to 4 m., and weighing 4 kgm. and more. Obviously, these plants have some good attachment or anchorage.

According to Fritsch (3, pp. 486–87) *Gracilaria confervoides* is a lithophyte or rock-attached plant, and "several arise from a large fleshy disc composed of closely compacted cortical threads; this, as well as the greater part of the erect thallus, persists from one season to the next." Ferguson Wood (9, p. 268) speaking of this species in Australia says in part, "The adherence to these (various) objects is feeble, sometimes by rhizoids, and sometimes by single attachments." Berthold (1, p. 432) says only that *Gracilaria* associated with *Phucagrostis* and *Caulerpa* covers the bottom of the Gulf of Naples "with a real turf, in quieter places of lesser depth, the substrate being muddy sand." G. Funk (4, p. 271) says only, "On the sandier parts of the inner Gulf (of Naples), in front of the Via Caracciolo, in 1–2 meters depth, the *Zostera*

[1] *Manuscript received in original form January 9, 1950, and, as revised, June 2, 1950.*

Contribution from the Department of Biology and Botany, The University of British Columbia, Vancouver, B.C.

[The August issue of Section C (Can. J. Research, C, 28 : 393-470. 1950.) was issued August 18, 1950.]

meadows appear to be quite replaced by floating (abgelöst) *Gracilaria confervoides*. It is at times an extraordinary thick stand that this alga can form." Again, Humm (5) says that great masses of *Gracilaria confervoides* occur in shallow muddy land-locked bays near Beaufort, N.C. He says that these masses are unattached and drifting, apparently sterile plants growing vegetatively.

None of the above writers excepting Wood mentions any means of attachment for the *Gracilaria* plants other than that described by Fritsch (3). Funk calls them "abgelöst", which I have taken to mean, loose in the water. Wood says they are always attached to some buried object such as a shell, tree cone, etc., either by "rhizoids" or "single attachments", and adds that the attachment is feeble.

In addition to the above references, Dr. C. K. Tseng, then of the Scripps Institute of Oceanography, La Jolla, in 1945 stated, in oral conversation with the present writer, that *Gracilaria confervoides* when anchored on sandy bottom had always been found to be attached by the usual discoid holdfast (Fritsch (3)) to some buried object, usually a pebble or shell. He expressed some astonishment at the sight of the method of anchoring described in this paper.

Finally, it should be stated that the present writer has never found sexual plants of *Gracilaria* that were not attached in the "conventional" manner described by Fritsch and Tseng as quoted. Observations and remarks to follow, where they concern the association of *Gracilaria* with annelid worms, apply strictly and solely to tetrasporic or sterile plants which, growing anchored and not free-floating, do indeed "in quieter places of lesser depth, the substrate being muddy sand, form a real turf." (Berthold (1, p. 432)). Elsewhere, they form open stands of clumps 3 to 4 m. tall at maximum.

When dug out with a shovel these clumps are almost invariably found to have the buried ends of their strands looped around the tube of an annelid worm, *Diopatra ornata* Moore. A somewhat similar case is mentioned for Australia by Wood (9, p. 268), but he speaks of "rhizoids" attached to "Turbellarian worm casts" which seems anomalous. The phenomenon now referred to is general on both sandy and gravelly beaches on the southeast coast of Vancouver island, and has not so far been noted north of about latitude 49° N., although both the plant and the animal occur far north of this, *Gracilaria* having been found by several persons, including myself, in latitude 54° 30′ N.

Observations

On one of the beaches above referred to, shown on charts as Boatswain's Bank in Satellite channel at the mouth of Cowichan Bay, the conditions are as follows: At the level of "zero" tides, i.e., the level of lowest normal low tides, or "Datum" of tide tables (2, p. 2), the beach is of sand firm enough to drive a motor car over it. From the 2-ft. tide level to minus 3 or 4 ft., it is a

nearly flat expanse about $\frac{1}{4}$ mile wide by 1 mile long ($\frac{1}{2}$ km. by 1.6 km.). The surface appears nearly bare of all life, with here and there slightly projecting tubes of annelid worms and the siphons of the giant clams, *Schizothaerus nuttallii* (Conrad), the horse clam, and *Panope generosa* Gould, the geoduck (pronounced "goeyduck", an Indian name). Infrequent cockles (*Cardium* species) and sand dollars, *Dendraster excentricus* (Escholtz), lie on the surface along with empty shells of various bivalves, and moon snails, *Polynices lewisii*. Except for these scattered objects, the surface is mostly smooth sand. Actually, there is just under the surface a massive population of annelid tube-worms, sand dollars, molluscs, crustaceans, and fishes, as will be described in other papers now in preparation.

In the part of the beach immediately around the zero tide level, *Gracilaria* plants become abundant. Just here, too, the subsurface is a dense meshwork of the intertwined tubes of annelids of three principal species. Two of these, *Telepsavus costarum* Claparède, about 3 mm. in diameter, and *Mesochaetopterus taylori* Potts, up to 10–12 mm. tube diameter and with a sandpaper-like surface to the tubes, are apparently not concerned in the anchorage phenomenon. The third species, *Diopatra ornata* Moore, has elastic, tough tubes up to 10–15 mm. diameter, rugose on the surface, and usually slanted into the sand to a depth of 5 dm. or more. This latter species, the principal anchor former, thus functions much like a "deadman" used in engineering to anchor a cable or stay. This anchoring of the alga by the worm tube was pointed out to the writer in 1944 by Dr. A. H. Hutchinson. After seeing the association of plant and animal many times, the writer was puzzled as to how the attachment of the alga, wrapped around and around the worm tube and cemented to its surface, could occur. It is not any matter of a single strand or a discoid holdfast, nor yet of Wood's "rhizoids", all described as being feebly attached or easily broken. Instead, the main strands of the clump, fully colored, and apparently normal, are wrapped around and around the tube, and so firmly cemented to it that it is impossible to detach the clump from its place without forcibly snapping off the strands at a distance from the tube. Often the worm tube breaks and the whole thing, anchor and all, is lifted by the collector, if the enwrapment is not buried more than a centimeter or two. Furthermore, the anchoring is so general an occurrence in the region that it seemed there must be some definite mechanism involved.

During the visits to the beaches on nights of zero tides in the winter of 1946–47, the following observations were made repeatedly. (These low tides in winter come near midnight, when the water temperature is usually about 4 to 5 °C.) At these times the *Gracilaria* plants break off in great numbers if there is a bit of wind. They wash in along the beach like great hanks of coarse hair 1 to 4 m. long, locally called Mermaid's Hair. At the same time the churning of the wavelets moves the sand to some extent and often bares 10 to 12 cm. of the nearly vertically placed ends of the worm tubes. As the tide rises it brings the rolling hanks of *Gracilaria* into contact with these projections, and with the siphons of the clams before mentioned. The strands of

alga are naturally bent around the projections on both sides, but the clam siphons are both slippery and flexible, also retractile. Hence there is no lengthy halting of the algal hanks at clam siphons but only at the rough, stiff worm tubes. On occasions when the waves were not too big, amplitude about 2 dm., a drift of sand was seen to form quickly on top of each halted hank of alga, at (and this is the crux of the process) only the stationary part in contact with the worm tube. In the course of 15 or 20 min. the whole thing, tube and all, was buried in a small drift of sand, leaving the free ends of the strands floating. By this time the water was deep enough so that the sand was no longer washed away by the wavelets, and being as aforesaid of a texture that is normally hard-packing, the drifted sand became quite hard and firm. A number of such drifts that had been observed to form were marked with stakes, and inspected at the next zero tides.

When next observed, the drifts were not evident but marked clumps of alga were still in place. A few of them were dug up and found to be wrapped around the worm tubes, as seen originally, but not cemented to them. On a subsequent visit the remaining clumps were examined and in each case the worm tube was found to have assumed a slightly more horizontal position and to have grown a little in length, judging by the region of enwrapment. The strands of *Gracilaria* were now cemented fast to the tube (after about four months). It was not possible to remove the alga from the tube easily without breaking the strands off short at the point or region of enwrapment. In addition to the original simple folding about the worm tube, the alga had also apparently grown around the tube by proliferating side branches. so that actually the tube, like all nonexperimental ones seen, was finally wrapped around and around by algal strands like a stake rolled in a fish net.

Discussion

As they grow, the proliferating side branches are probably closely appressed to the tube surface by pressure of the surrounding sand, and become cemented to the surface of the worm tube. They are at last almost if not quite entirely embedded in the material of the tube. The final result is the firm implantation and anchoring of a fresh clump of *Gracilaria*, through the mechanical coaction of the plant and animal under the influence of the physical factors of the environment. That there is also a strictly biological action at work to provide the cementing and enwrapping effects can hardly be doubted, but to elucidate it will require careful study in a laboratory equipped with large specially designed tanks, with a plenitude of running sea water.

It is not at all certain that there is not some cementing substance present other than the obvious tube-forming secretion of the *Diopatra* worm. It is notorious with collectors of *Gracilaria* on the B.C. coast that the lower strands of dense clumps are often cemented together with some tough, elastic substance resembling variously the material of the *Diopatra* tubes or simply epidermal

mucus. These cemented strands form tubes which are inhabited usually by large amphipods, but often by small polychaete worms. The cemented strands are nuisances because they dry very slowly, often decomposing first. Besides, the deceased inhabitants give the dried material a distinctly "fishy" aroma, not commercially desirable.

Ecological Implications

Various authors, especially Rice (6, p. 298), Shelford (7, pp. 276–78, 283–84, 309), and Shelford and Towler (8, pp. 60, 65), have referred to algae as components of the communities in this region, but have been inclined to dismiss them as of only seasonal importance. Shelford particularly (7, pp. 283–84), says they are not present throughout the year and often constitute seasonal societies only. The present writer, having studied them throughout the year for the past six years, would disagree with this conclusion to some extent, as only a few species of algae have been found to be annuals. Even the bull kelp, *Nereocystis*, persists in quantity. And as described in this paper, it is in the middle of the winter that *Gracilaria* appears to have a distinct coaction with at least one conspicuous animal. However, it is quite as Shelford says in the same place (7), the animals sheltering in the clumps are vastly increased in numbers in the summer, this being when the perennating algal clumps reach their greatest growth. This is quite comparable to the seasonal effect of vegetation of grasslands and deciduous forests upon insect populations. It is suggested that studies of marine communities where this or plants of similar life form (e.g. *Cystophyllum geminatum*) occur, should take the presently described type of coaction into account as being a normal phenomenon to be looked for as providing vegetative multiplication and spread of attached stands of algae, needing neither spores nor sexual plants. It is not suggested that the cementing effect referred to is due to any secretion of the plants, but rather otherwise.

Acknowledgments

The problem of the attachment of *Gracilaria* plants first came to the attention of the writer while he was working under the direction of Dr. A. H. Hutchinson, Head of the Department of the University of British Columbia of which this writer is a member. Professor Hutchinson made the discovery of the previously unreported method of anchoring in the spring of 1944. The preliminary observations which led to a closer study of the mechanics of the matter were made while the writer was employed as a field worker for the B.C. Research Council. Later, field experiments were conducted by the author as a member of the Department of Biology and Botany of this University, travelling expenses being contributed by the Krim-Ko Company of Chicago. The three species of annelids referred to and discussed were kindly identified by Mr. and Mrs. C. Berkeley of Nanaimo.

References

1. BERTHOLD, G. Ueber die Vertheilung der Algen im Golf von Neapel, etc. Mitt. Zool. Sta. Neapel, 3 : 393-536. 1882.
2. DEPARTMENT OF MINES AND RESOURCES, CANADA. Tide tables for the Pacific Coast of Canada, 1948. Tidal Pub. No. 10, Surveys and Engineering Branch, Ottawa. (N.B. The 1949 and later editions of this publication have a change in the datum of the tide level at certain stations.) 1948.
3. FRITSCH, F. E. The structure and reproduction of the algae. II. Cambridge University Press, London. 1945.
4. FUNK, G. Die Algenvegetation des Golfs von Neapel. Pub. Staz. Zool. Napoli, 7 (Supplement). 1927.
5. HUMM, H. J. Agar resources of the South Atlantic and East Gulf Coasts. Science, 100 (2593) : 209-212. 1944.
6. RICE, LUCILE. Some marine biotic communities of the Pacific Coast of North America. Part I. Dynamics and extent of bottom and shore communities. 1. *Balanus-Littorina* Biome. Ecol. Monographs, 5 : 293-304. 1935.
7. SHELFORD, V. E. Some marine biotic communities of the Pacific Coast of North America. Part II. General Survey of the communities. Ecol. Monographs, 5 : 249-292. 1935.
8. SHELFORD, V. E. and TOWLER, E. D. Animal communities of the channel and adjacent areas. Pubs. Puget Sound Biol. Sta. Univ. Wash. 5 : 33-73. 1925.
9. WOOD, E. J. FERGUSON. The sources of agar in Australia. J. Australian Council Sci. Ind. Research, 18(3) : 263-275. 1945.

TWO NEW OUTBREAKS OF *PHOMOPSIS LOKOYAE* IN BRITISH COLUMBIA[1]

By G. P. Thomas[2]

Abstract

Young Douglas fir in British Columbia sustained damage from *Phomopsis lokoyae* Hahn during the winter of 1948–49. Damage in the form of annual stem and branch cankers, leader die-back, and stem girdling was confined to trees, or parts of trees, 4 in. in diameter or less. Fungus activity was confined to Douglas fir bark and cambium tissues for the single dormant season. Outbreaks of the fungus are recurrent in British Columbia, reaching epidemic proportions only at irregular intervals.

Introduction

Damage to young Douglas fir (*Pseudotsuga taxifolia* (Poir.) Britt.), associated with *Phomopsis lokoyae* Hahn in the form of stem and branch cankers and leader die-back, was observed in two widely separated localities in British Columbia during the early summer of 1949. Similar damage to Douglas fir saplings, associated with the same fungus, has been reported previously by Boyce (1, 2) and Hahn (3). While both the causal agent and the damage to host are identical with previous descriptions, it is believed that one of the British Columbia outbreaks represents a significant departure from the restricted distribution of the fungus as recorded by Boyce (1, 2) and Hahn (3).

Host and Locality

Previous to the 1949 observations the fungus was known to occur in southern Oregon, northern California, and southwestern British Columbia. In this range it was localized in extent and occurred on the Pacific Coast, or green, form of Douglas fir. Of the two 1949 observations one was located at Haney, in the lower Fraser Valley, the damage occurring to Pacific Coast Douglas fir. The second outbreak occurred some 300 miles distant, well within the Interior Forest, and caused damage to Rocky Mountain, or blue, Douglas fir. This occurrence represents a departure from the restriction of the fungus to Pacific Coast Douglas fir.

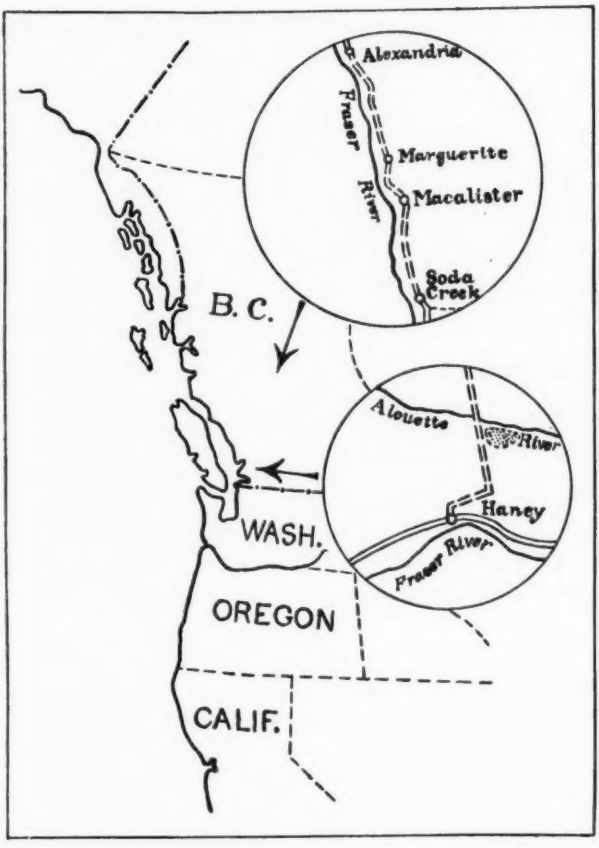
The Haney outbreak was small, covering only about 10 ac. The second outbreak, centered between Marguerite and Macalister and extending north to Alexandria and south to Soda Creek, covered an area parallel to the highway for a distance of 23 miles.

In the light of observations made by Boyce (2) that the disease in Oregon and California was confined to trees on poor sites it can only be said that the disease in British Columbia was not found on the highest site land. The Haney area was better-than-average site land while the second area was low site land.

[1] *Manuscript received June 1, 1950.*

Contribution No. 1029 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] *Assistant Forest Pathologist, Dominion Laboratory of Forest Pathology, Victoria, B.C.*



TEXT-FIG. 1. *Outline map indicating the location of two outbreaks of Phomopsis lokoyae on Douglas fir in British Columbia in 1949.*

Stem girdling of young Douglas fir.

FIG. 1. *A 4-in sapling with multiple stem cankers extending from five feet to eight feet. All tissues above the lowest point of girdling are killed.*

FIG. 2. *A stem canker on a 3 in. sapling. Note the smooth surface characteristic of young cankers and the formation of callus ridges at the edge of the canker. Girdling of this tree is complete as evidenced by desiccated tissues above the canker.*

FIG. 3. *Complete girdling of a 2 in. stem. Normal green foliage and healthy tissues persist below the lowest point of infection. Resinosis was evident in some cases but cannot be regarded as a symptom of the disease.*

FIG. 4. *An old stem canker showing characteristic delimitation of affected area by callus tissues. This canker has subsequently girdled the tree.*

PLATE I

PLATE II

1

2

3

Damage

Damage was confined to trees of sapling size, generally 4 in. d.b.h.o.b.* or less. If larger trees were affected the damage was restricted to the tops, rarely extending down the bole below a point 4 in. in diameter.

As in the case of *Phomopsis Pseudotsugae* Wilson, three distinct types of lesions were observed (4), viz. a definite die-back of leaders and twigs, a typical girdling wherein the death of tissues beyond the girdled area occurs more slowly than in the case of die-back, and a definite canker of limited growth on the bole and, to a lesser extent, on branches.

Dying back of leaders and small branches was quite common. The almost typical bark anthracnose condition did not extend, in most cases, into tops greater than 1 in. in diameter. There was no evidence of leader recovery by the end of the summer, hence this form of damage may be considered permanent.

Girdling of branches and the main stem, with subsequent dying of tissues beyond the point of girdling was common. The maximum diameter at which girdling took place was 4 in. and the average diameter at which girdling occurred was approximately 2 in. Dying of tissues beyond the point of girdling occurred much less rapidly than in cases of leader die-back.

Stem and branch cankers, unattended by top or branch killing, were less in evidence than those lesions causing death of tops and branches. However, close inspection of many trees revealed one to several cankers per tree. Such cankers exhibited considerable variation in outline and extent but, in general, were elliptical, sunken when first formed, and delimited by a raised callus tissue when older. Old cankers are made conspicuous by a pulling away of dead tissues from callus growth. In all cases cankers were centered by a twig or small branch which, apparently, provided the means of inoculation into the bole or branch.

Damage and effect were similar in both forms of Douglas fir. Stem girdling was considered to be the most serious form of damage, particularly in small trees. Leader and twig die-back was less common and therefore less serious. Stem and branch cankers that did not girdle the host were of only minor importance.

* *Diameter at breast height (4.5 ft.) outside bark.*

Branch and stem cankers of Douglas fir.

FIG. 1. *A branch canker on the under side of a $\frac{3}{4}$ in. branch. Note that the canker has been arrested by callus formation. This canker will eventually become healed over.*

FIG. 2. *Two stem cankers on a 4 in. fir sapling. Note that each canker is centered by a twig or small branch and that the formation of callus has begun at the margins of the cankers.*

FIG. 3. *An old stem canker on a fir sapling. This canker did not cause girdling as is shown by complete callusing of the cankered area. Note the dead cankered tissue suspended by a branch. (Photograph by J. E. Bier.)*

Boyce (2) considers the disease to be one of long standing in Oregon and California and although it is capable of becoming locally epidemic, it does not cause serious losses even under conditions favorable to its development.

Causal Agent

Phomopsis canker of Pacific Coast and Rocky Mountain Douglas fir is directly associated with *Phomopsis lokoyae* Hahn, one of the Fungi Imperfecti. Damage attributed to the fungus was first noted by Boyce in 1930 in Oregon and California, and the causal fungus was isolated and described by Hahn in 1933.

Inoculation apparently occurs through young shoots during late summer. Infection spreads into the bole, or larger branches, through the bark and cambium, the sapwood remaining free from attack. The fungus ramifies in the bark and cambium throughout the winter and spring following inoculation. At the start of the growing season infected tissues become delimited from healthy tissues by callus growth and the fungus ceases to spread. If girdling has not occurred by this time lesions will eventually heal over and no permanent damage will result.

Minute black pycnidia issue through the bark of diseased areas during the summer months. They are liberally distributed over the surfaces of killed-back leaders and stem and branch cankers. Pycnidiospores are produced and are disseminated, probably by wind, during the summer.

According to Boyce (2), the development of the disease appears to be substantially favored by periods of heavy summer rainfall which are followed by protracted periods of drought. Both areas in British Columbia experienced heavy and consistent rainfall during the summer of 1948 but in neither locality was a period of drought in evidence, even for a short period. It may be worthy of note that both areas suffered an unusually long and severe winter in 1948–49.

Summary

Phomopsis lokoyae Hahn was found associated with leader die-back, stem girdling, and stem and branch cankers on Pacific Coast and Rocky Mountain Douglas fir in two widely separated localities in British Columbia in 1949. Damage was confined to trees of sapling size, rarely occurring on trees, or parts of trees, greater in diameter than 4 in. The fungus is active for a single dormant season following inoculation and is restricted to bark and cambium tissues. Unless leaders are killed back or stems are girdled during the one season of fungus activity affected trees will recover and no serious permanent defect will result. The disease is believed to be favored by periods of heavy summer rainfall followed by a period of protracted drought. Although recurrent outbreaks may be anticipated the disease is not thought to constitute a serious threat to sapling Douglas fir in the Pacific northwest.

References

1. BOYCE, J. S. A canker of Douglas fir associated with *Phomopsis lokoyae*. J. Forestry, 31 : 664-672. 1933.
2. BOYCE, J. S. Forest pathology. McGraw-Hill Book Company, Inc., New York, Toronto, London. 1948.
3. HAHN, G. G. An undescribed *Phomopsis* from Douglas fir on the Pacific coast. Mycologia, 25 : 369-375. 1933.
4. WILSON, M. and HAHN, G. G. The history and distribution of *Phomopsis pseudotsugae* in Europe. Phytopathology, 19 : 979-992. 1929.

THREE YEARS OF EVAPOTRANSPIRATION AT TORONTO[1]

By Marie Sanderson[2]

Abstract

An experiment to measure the daily potential evapotranspiration from vegetation-covered soil surfaces was conducted in Toronto during the 1947, 1948, and 1949 growing seasons. The potential evaporation from moist bare soil was also measured and compared with the evaporation from dry soil. Results indicate that the Thornthwaite formula for computing potential water loss is valid at this latitude for both vegetation-covered and moist soil. When drying of the surface soil occurred, the water loss was diminished.

To determine whether precipitation is sufficient for the needs of the vegetation in any locality, it is necessary to know how much water will evaporate and transpire if it is always available in optimum quantity. This important climatic concept of "potential evapotranspiration" was used by C. W. Thornthwaite, Director of the Johns Hopkins Laboratory of Climatology, as the basis of his recent climatic classification (6); and he developed a formula for computing the potential evapotranspiration from the meteorological statistics of temperature and length of day. To verify the formula and to obtain daily measurements of the little-known element of potential evapotranspiration, Thornthwaite also designed an experiment to permit its measurement under natural conditions (7). The principle of the experiment is to keep a vegetation-covered area constantly supplied with water by subirrigation and to measure the daily water use.

Installations of evapotranspirometers have been made in New Jersey (2), Mexico (1), Toronto and Kapuskasing in Ontario, and Norman Wells in the Northwest Territories (3). An earlier article in this Journal described the initiation of the experiment in Toronto in 1947 (4). A diagrammatic sketch illustrated the principle of the evapotranspirometer, and a photograph showed the four units in operation. The daily readings for the 1947 season from June 25 to Nov. 14 were also published.

Readings from the four evapotranspirometers were continued during the 1948 and 1949 season, but the unavoidable termination of the experiment in November, 1949, made it advisable to prepare a report of the three years' operations. The experiment will be continued in the 1950 season at a new location in southern Ontario.

Changes in Procedure and Equipment

The original purpose of the experiment was to test the Thornthwaite formula by measuring the daily evapotranspiration from natural grass cover. However, since it is commonly believed that different species of grass transpire

[1] *Manuscript received May 18, 1950.*
Contribution from the Department of Physiography, Ontario Research Foundation, Toronto, Ont.

[2] *Research fellow.*

different amounts of water, two of the tanks were sown with drought-resistant crested wheat grass, and two with moisture-loving timothy grass. The 1947 season's results showed no difference in the measured water losses from the two species of grass. Consequently, in 1948, it was decided to investigate the differences in water loss from the two extremes of cover found in agricultural land: luxuriant grass vegetation and bare soil. The timothy grass was left in two evapotranspirometer tanks, called A and B in this report, and was well fertilized, while the crested wheat grass was removed and the bare soil exposed in the remaining tanks, C and D. A further modification was made in one of the bare soil tanks in 1949, when the water level was raised from two ft., the level in the other tanks, to 1 ft. below the surface to ensure moist surface soil at all times. The experimental procedure followed each year on the four tanks is indicated below:

	Tank A	Tank B	Tank C	Tank D
1947	Timothy— no fertilizer	Timothy— no fertilizer	Crested wheat grass— no fertilizer	Crested wheat— no fertilizer
1948	2 lb. "	2 lb. "	Bare soil	Bare soil
1949	3½ " "	3½ " "	" "	" " (Water table raised 1 ft.)

During the 1948 and 1949 seasons, equal amounts of commercial fertilizer were applied to tanks A and B when the grass showed signs of yellowing. During all three seasons the grass in the tanks was cut at the same time as the surrounding grass, since a similar environment inside and outside the tanks is necessary for true values of potential evapotranspiration. For the same reason the grass in the surrounding areas was irrigated during dry periods to ensure uniform growth. Fig. 1 shows the installation in 1949, with bare soil tanks C and D in the right foreground, and timothy tanks A and B scarcely visible in the grass-covered environment in the right background.

There were two minor changes in equipment since the writing of the earlier article. In 1949 an improved type of intake tank was installed (seen on the left in Fig. 1), with an inside area 1/50 that of the evaporating surface of the transpirometers. A glass gauge, with scale graduated in mm. mounted on the outside of the tanks, simplified the taking of the readings, and made the daily filling of the reservoirs with water unnecessary. Because of the importance of adequate sampling of the rainfall, an additional rain gauge was installed in 1948.

Presentation of Data

Detailed daily records were obtained. Readings for the 1948 season were from June 13 to Nov. 30; and for the 1949 season from May 2 to Nov. 22. In both years, readings were begun in spring as soon as experimental changes allowed and continued in the fall until low temperature caused the freezing of the water in the reservoirs. For both the 1948 and 1949 seasons, the daily readings included temperature, precipitation, and the intake and overflow expressed as depth of water (in mm.) over the evaporating surface.

The daily values of water loss or potential evapotranspiration are thus obtained by the formula

$$E = I + P - O$$

where E is evapotranspiration, I is intake, P is precipitation, and O is overflow.

The data on water loss will be analyzed in two sections; (*a*) evapotranspiration from grass vegetation, and (*b*) evaporation from bare soil. Finally, the amount of grass produced each year on the vegetation-covered transpirometers will be discussed.

Evapotranspiration from Grass

The daily figures of measured evapotranspiration from A and B can be compared with the daily figures of potential evapotranspiration computed from the Thornthwaite formula. However, the comparison is not always valid. On days when precipitation occurs, the apparent value of E is greatly exaggerated, since an unknown quantity of the precipitation does not become overflow but is held by the soil mass and contributes to the evapotranspiration of succeeding days. For this reason, the values of E for the day or two following precipitation are unnaturally low. The highest measured loss for a 24 hr. period, uncomplicated by precipitation, was 9.7 mm. and occurred on Aug. 25, 1948. There were a few days in both seasons when measured evapotranspiration amounted to more than 7 mm., but the highest computed value of E was 5.7 mm. in 1948 and 7.3 mm. in 1949.

The effect of precipitation on the daily readings of water loss can be offset by obtaining weekly or 10-day averages, which can be fairly compared with computed averages. Such 10-day average values for the three complete seasons of observation are shown in Fig. 2. The solid black line represents measured, and the dashed line computed evapotranspiration. The similarity between the two is most gratifying. During one period only, that of May-June, 1949, was the measured E consistently greater than the computed. The dotted line on each graph represents average potential evapotranspiration obtained from the 100-year average Toronto temperatures. During the major part of all three seasons the curves of measured and computed E bear a marked resemblance to the curve of long-term average evapotranspiration.

PLATE I

FIG. 1. *Evapotranspirometer experiment, Toronto, 1949. Two tanks, right foreground—bare soil. Two tanks, right background—timothy grass.(Rims of tanks not visible in sod.)*

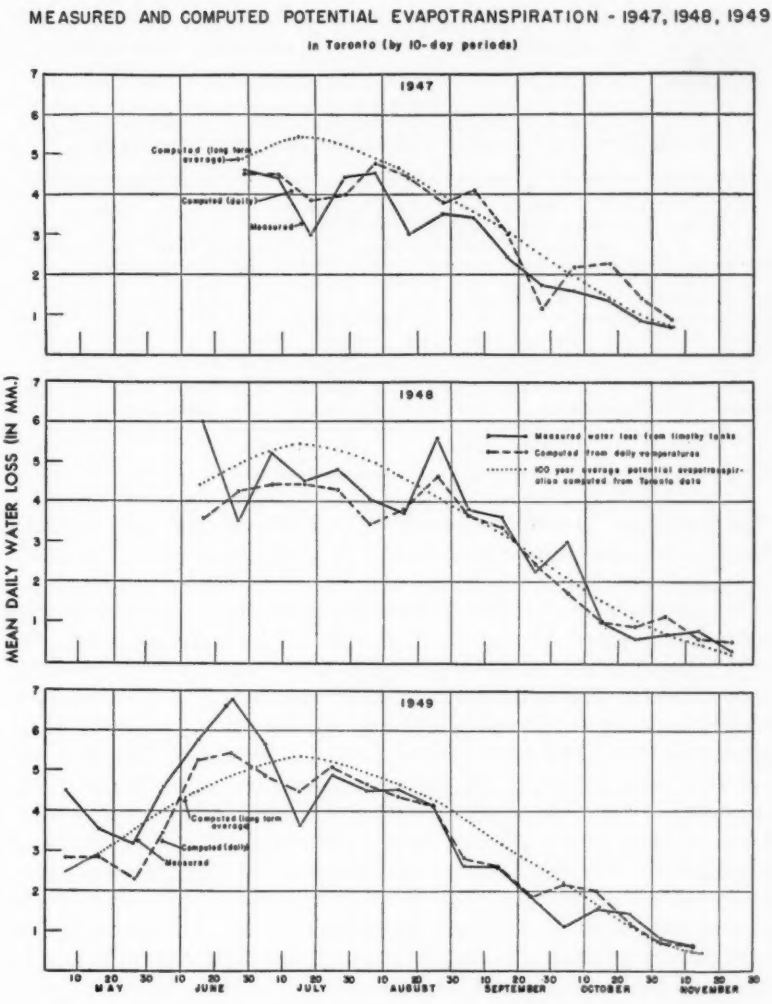


FIG. 2. *Measured and computed potential evapotranspiration 1947, 1948, 1949 (averaged for 10-day periods).*

×————× *measured water loss from timothy tanks.*

o — — — o *water loss computed by Thornthwaite formula from daily temperatures.*

• . . . . . • *100- year average potential evapotranspiration computed from Toronto data.*

As a summary, Table I shows the total monthly water losses from the grass-covered surfaces of the evapotranspirometer tanks, A and B (and in 1947, C and D also) and compares them with the total computed water losses.

TABLE I

MONTHLY TOTALS OF POTENTIAL EVAPOTRANSPIRATION FROM GRASS (IN CM.)

	1947		1948		1949	
	Measured	Computed	Measured	Computed	Measured	Computed
May					11.2	7.9
June	(6 days) 2.6	2.9	(18 days) 8.6	7.2	16.3	14.1
July	12.4	13.0	14.8	13.4	15.0	14.8
August	11.5	13.4	14.2	12.2	13.2	13.3
September	8.0	8.6	9.6	9.4	6.2	7.0
October	4.0	6.1	4.8	3.7	4.4	5.5
November	(14 days) 1.0	1.0	1.6	2.2	(22 days) 1.4	1.0
Total	39.5	45.0	53.6	48.1	67.7	63.6

The similarity between the monthly totals of measured and computed potential evapotranspiration is striking. In only three months during the three seasons was there a difference of more than 2 cm. between the measured and the computed totals. The close relationship between the seasonal totals for the three years is also apparent. In 1947, the total measured water loss was 12% less than the computed; in 1948, 11% greater, and in 1949, 6% greater. During July and August in 1948, and during May and June in 1949, the measured water loss was higher than the computed, but the opposite was true in 1947. During the cooler months of September, October, and November, measured water loss was less than computed in 1947 and 1949 and greater in 1948. It is apparent, then, that the Thornthwaite formula did not consistently overestimate evapotranspiration for specific months of the year, nor consistently underestimate it for others. The low evapotranspiration of 1947 may be due to the fact that the grass was planted in May of that year, but not fertilized. Thus the sparse growth in the early part of the season and the prevalence of dead grass during the latter part of the period of observation doubtless reduced transpiration. Differences between seasons are perhaps due to meteorological causes, but experimental data at the present time are not sufficient to warrant further analysis.

To permit comparison, Fig. 3 shows accumulated measured and computed evapotranspiration during the period of observation common to all three years, June 24 to Nov. 14. The computed water losses for the three seasons, represented by the dashed lines, appear surprisingly similar, while, as was seen in Table I, measured evapotranspiration was lowest in 1947 and highest in 1948.

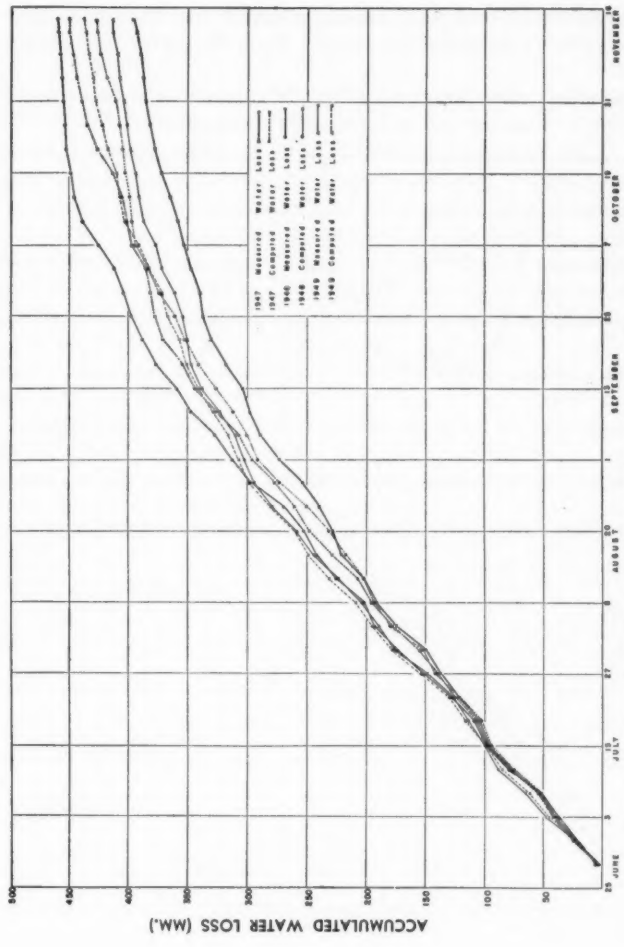


FIG. 3. *Accumulated water losses from timothy grass, compared with computed water losses, for period of observation common to 1947, 1948, 1949.*

X———X *1947 measured water loss;* X- - - - - -X *1947 computed water loss.*

o———o *1948 measured water loss;* o - - - - - - o *1948 computed water loss.*

^———^ *1949 measured water loss;* ^ - - - - - - ^ *1949 computed water loss.*

Evaporation from Bare Soil

When the vegetation was removed from tanks C and D in 1948 and the bare soil exposed, it was soon found that the surface of the soil became dry on hot, sunny days and the daily water loss was 1 to 3 mm. less than that from the grass surfaces. On cooler days in autumn, with lower evaporation, the surface remained moist and the readings of water loss from bare soil were comparable to those from grass vegetation. Since the surface soil often dried

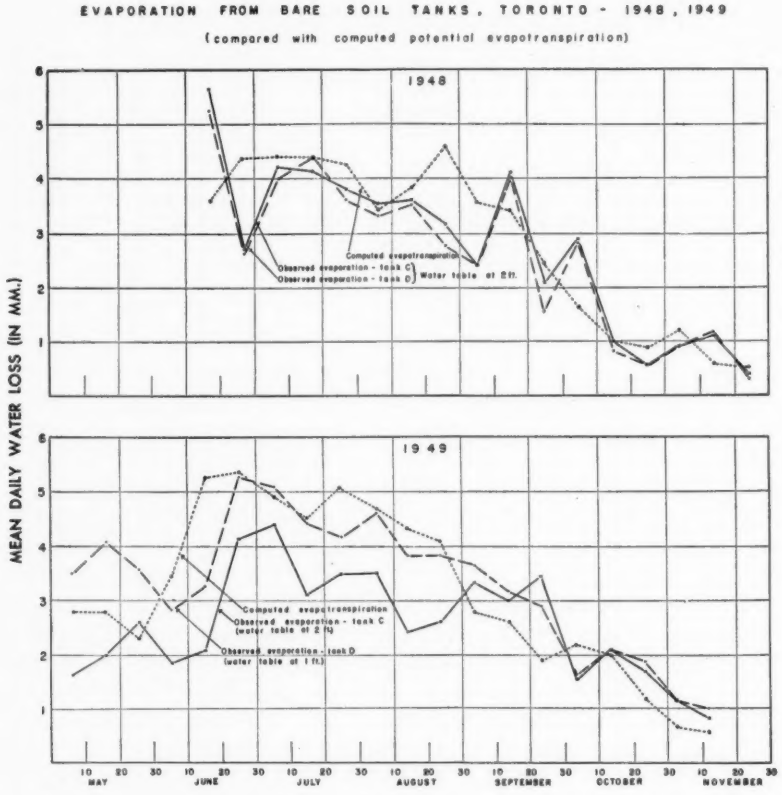


FIG. 4. *Evaporation from bare soil tanks, Toronto 1948–1949. (Compared with computed potential evapotranspiration.)*

1948 ×———× *evaporation from tank C (water table at 2 ft.).*
•— — —• *evaporation from tank D (water table at 2 ft.).*
o- - - - - -o *computed evapotranspiration.*

1949 ×———× *evaporation from tank C (water table at 2 ft.).*
•— — —• *evaporation from tank D (water table at 1 ft.).*
o- - - - - -o *computed evapotranspiration.*

out, the figures of water loss here cannot be said to represent "potential" evaporation, but rather the actual water loss that would occur from natural fallow soil.

During 1948, an attempt was made to keep the soil moist by surface sprinkling in July. When this was done, it was noted that the daily water losses from the bare soil were similar to those from the timothy tanks A and B. Also, on days when precipitation occurred, the evaporation from C and D was the same or slightly more than the grass evapotranspiration. In 1949, the water table in tank D was raised 1 ft., and the surface soil remained moist throughout the season. The daily readings of water loss here represent potential evaporation and are of the same magnitude as those from the grass surfaces.

Fig. 4 shows the evaporation averaged by 10-day periods for bare soil for the 1948 and 1949 seasons. The record for tank C is represented by a solid black line on the graphs, that of tank D by a broken line, while the open circles connected by the dotted line represent the 10-day average values of computed evapotranspiration. During the 1948 season the readings from the two tanks were very similar and the comparison with computed E was good, with the exception of the dry, hot period of mid-August to early September. The graph of the 1949 season shows that the seasonal trends in water loss are the same for tanks C and D, although during the May to August period, the daily readings from C, with lower water table, were consistently about 1 mm. below those from D. From September to November the two tanks acted similarly and the water losses for both were higher than the computed water losses. It is interesting to note in Fig. 4 that the evaporation curves from C and D follow the same pattern as the curve of computed water loss, with a time lag of a week to 10 days. No satisfactory explanation can be offered for this phenomenon.

The monthly totals of bare soil evaporation are given in Table II and compared with measured and computed potential evapotranspiration.

TABLE II

MONTHLY TOTALS OF BARE SOIL EVAPORATION COMPARED WITH POTENTIAL EVAPOTRANSPIRATION (IN CM.)

Month	1948				1949			
	Bare soil C	Bare soil D	Grass vege-tation	Com-puted	Bare soil C	Bare soil D	Grass vege-tation	Com-puted
May					6.3	11.2	11.2	7.9
June	(18 days) 7.6	7.4	8.6	7.2	8.0	11.3	16.3	14.1
July	12.4	12.1	14.8	13.4	11.3	14.1	15.0	14.8
August	10.8	10.0	14.2	12.2	9.2	12.7	13.2	13.3
September	8.6	8.1	9.6	9.4	8.7	8.9	6.2	7.0
October	4.6	4.4	4.8	3.7	5.9	6.1	4.4	5.5
November	2.2	2.1	1.6	2.2	(22 days) 2.0	2.3	1.5	1.0
Total	46.2	44.1	53.6	48.1	51.4	66.6	67.8	63.6

For the 1948 season, the average evaporation from C and D was 45.1 cm., 84% of the potential evapotranspiration from tanks A and B and 94% of the computed evapotranspiration. In 1949, a season with less precipitation during the warmer months, evaporation from C was only 76% of the evapotranspiration from the vegetated tanks and 81% of the computed. Total evaporation from the moist soil in tank D was 98% of the measured evapotranspiration and 5% greater than the computed water loss. Fig. 5 compares the 1949 accumulated water losses from the bare soil tanks C and D and the vegetation covered tanks A and B with potential evapotranspiration computed from the Thornthwaite formula. The close similarity between computed water loss and that measured from moist soil can especially be noted.

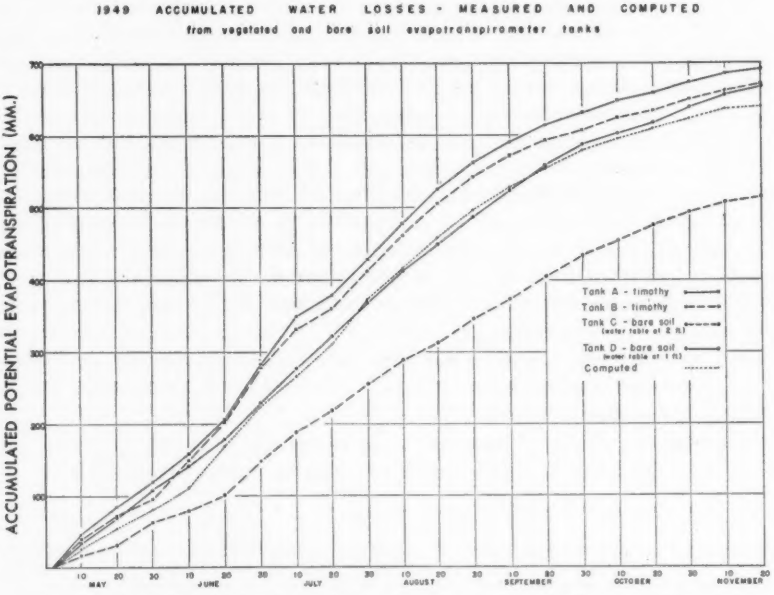


FIG. 5. *Accumulated water losses, both measured and computed, from vegetated and bare soil evapotranspirometer tanks.*

×———× *Tank A—timothy grass.*
×— — —× *Tank B—timothy grass.*
o— — —o *Tank C—bare soil (water table at 2 ft.).*
o———o *Tank D—bare soil (water table at 1 ft.).*
•– – – –• *computed potential evapotranspiration.*

Grass Weights

To observe the relationship between the potential evapotranspiration from a grass covered area 25 ft. square and the amount of dry matter produced on that area, the grass in tanks A and B was periodically cut, air-dried, and weighed.

The weights are shown in Table III.

TABLE III

AIR-DRY WEIGHTS OF TIMOTHY GRASS (IN GM.) CLIPPED FROM EVAPOTRANSPIROMETER TANKS (WITH AREAS OF 25 SQ. FT.)

1947		1948		1949	
Date	Weight	Date	Weight	Date	Weight
				May 19	156
				June 3	126
		June 28	130	June 16	153
		July 6	78	June 23	102
		July 19	102	July 11	142
		Aug. 5	206	Aug. 3	328
July 22	63	Aug. 11	99	Aug. 10	95
Aug. 11	95	Aug. 20	111	Sept. 1	174
Sept. 10	61	Aug. 31	94	Sept. 21	177
Oct. 3	54	Sept. 22	130	Oct. 7	118
		Oct. 19	163		
Total	273	Total	1113	Total	1571

In 1948 and 1949 the first cutting is omitted since it represented growth during a period when the evapotranspiration was not measured. From the measured evapotranspiration during the same periods, the amount of water required to produce 1 gm. of dry weight can be obtained. In 1947 this was 1.44 mm., in 1948, 0.45 mm., and 0.37 mm. in 1949. The addition of fertilizer seemed to increase the efficiency of the water used in producing dry matter, since the corresponding amounts of fertilizer added to each tank were: in 1947—0, in 1948—2 lb., and in 1949—3.5 lb. However, apparently the weight of dry matter produced, or the density of the vegetation cover, had no effect on the amount of water loss, as can be seen below for periods of similar water use.

Period	Weight of grass (gm.)	Water loss (mm.)	
		Measured	Computed
1947—July 22 to Oct. 3 (74 days)	210	236	266
1948—July 19 to Sept. 22 (66 days)	640	276	253
1949—July 11 to Sept. 21 (72 days)	774	271	285

Summary

The three years' results from the Toronto evapotranspirometers verify the Thornthwaite formula for computing potential evapotranspiration at this latitude. Measured water loss closely approximated computed water loss regardless of the species of grass, the density of growth, or the application of fertilizer. But the application of fertilizer greatly increased the amount of

dry matter produced per millimeter of water loss. The formula did not consistently overestimate potential evapotranspiration for any one month or any one season during the three years, nor underestimate it for any other. There were definite periods when measured water losses were higher or lower than those computed from the formula, but the explanation of these discrepancies must await the further collection of data.

Evaporation from bare soil with a water table similarly 2 ft. below the surface was considerably less than that from a grass-covered surface, for periods of high temperature and high evapotranspiration. During this time, the amount of water loss from the bare soil increased with increased precipitation. During periods of lower temperature and less water loss, the evaporation from bare soil closely resembled both the evapotranspiration from grass-covered soil and that computed from the Thornthwaite formula. The evaporation from bare soil kept constantly moist by a high water table bore a remarkably close correlation with the computed water loss.

The verification of the Thornthwaite formula at the Toronto latitude, combined with the indication from similar experiments that the values of potential evapotranspiration it provides are also dependable at high latitude, lend further support to the application of the Thornthwaite classification to the whole of Canada (5).

References

1. CONTRERAS ARIAS, A. La Medida de la Evapotranspiracion, como Base para la solucion de Problemas Agricolas Importantes. Chapingo, 21 : 16-22. 1948.
2. THE JOHNS HOPKINS UNIVERSITY LABORATORY OF CLIMATOLOGY, Seabrook, New Jersey. Interim Repts. Nos. 4, 5, and 7.
3. SANDERSON, M. The measurement of potential evapotranspiration. Norman Wells, N.W.T. To be published.
4. SANDERSON, M. An experiment to measure potential evapotranspiration. Can. J. Research, C, 26 : 445-454. 1948.
5. SANDERSON, M. The climates of Canada according to the new Thornthwaite classification. Sci. Agr. 28 : 501-517. 1948.
6. Thornthwaite, C. W. An approach toward a rational classification of climate. Geograph. Rev. 38 : 55-94. 1948.
7. WILM, H. G., THORNTHWAITE, C. W., *et al.* Report of the committee on evaporation and transpiration 1945–46. Trans. Am. Geophys. Union, 27 : 720-725. 1946.

EFFECT OF PASMO DISEASE ON SEED YIELD AND THOUSAND KERNEL WEIGHT OF FLAX[1]

By W. E. Sackston[2]

Abstract

The pasmo pathogen sporulated freely on potato dextrose agar containing yeast extract. Spores suspended in water with gelatin as a spreader–sticker were sprayed onto flax plants at different stages of growth in field plots. Heaviest infections of pasmo resulted from inoculations at the flowering stage, lighter infections from inoculations on seedlings, and lightest infections from inoculations on ripening plants. Diluting the concentration of spores in the inoculum reduced disease intensity. The four flax varieties in the tests differed in reaction to pasmo. In decreasing order of susceptibility they were: Viking, Redwing, Royal, and Crystal. Heavy infections of pasmo caused premature ripening and reduced the seed yield and weight per thousand kernels of all four varieties. The effects of pasmo infection on seed yield and kernel weight were similar to those caused by a hot, dry climate, and by flax rust. Seed yield and kernel weight were reduced most markedly by inoculation at the flowering stage, less severely by inoculation at the seedling stage, and least of all by inoculation at the time of ripening. Seed yield and kernel weight from plots inoculated when the plants were ripening did not differ significantly from the uninoculated checks. Highly significant positive correlations between the data for seed yield and kernel weight indicated that much of the loss in yield resulted from a reduction in the size of individual seeds.

Introduction

Flax production in Western Canada increased greatly during the war years in response to a demand for oil seeds. This increase was particularly marked in Manitoba, where the total yield of flax rose from 425,000 bushels in 1939 to 2,800,000 bushels in 1945 (42, 43). The demand for flaxseed continued in the immediate postwar years. Good prices and high average yields, and consequent high cash returns per acre, resulted in the Manitoba flax acreage being raised from 284,000 in 1943, the wartime high, to 556,000 in 1947, and 960,000 in 1948 (43). Dry weather and low yields in western Saskatchewan, formerly the main flax area, speeded the eastward shift of flax production and made Manitoba and the adjacent portion of Saskatchewan the major flax-producing area in Canada. When flax supplies exceeded demand in 1948, guaranteed prices were reduced drastically. The resulting drop in flax seeding in Western Canada in 1949, like the more gradual increase during the war, was most striking in Manitoba, where the acreage fell from 960,000 in 1948 to 134,000 in 1949 (43).

The growing economic importance of flax in Manitoba and eastern Saskatchewan attracted attention to diseases of flax in that area for two main reasons. First, the larger acreages seeded to flax and the introduction of the crop into new districts made it easier for pathogens to build up inoculum, to survive from year to year, and to spread from diseased stubble to plants growing in

[1] *Manuscript received May 5, 1950.*

Contribution No. 1025, Division of Botany and Plant Pathology, Department of Agriculture, Ottawa, Canada. Part of a thesis presented in April, 1949, to the University of Minnesota in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] *Associate Plant Pathologist, Dominion Laboratory of Plant Pathology, Winnipeg, Canada.*

nearby fields. Second, the greater money value of each bushel of flaxseed resulted in heightened appreciation of losses caused by disease. As very little was known about diseases affecting flax in Western Canada, it was decided in 1944 to determine their distribution and the organisms responsible, and to investigate certain of them experimentally.

When the flax price fell and acreage was reduced, disease still continued to be an important factor in the economic production of the crop. The monetary loss per acre from disease had decreased, but the lower margin of profit associated with reduced prices made it imperative that production be as efficient as possible. These considerations justified continuation of the investigations begun in 1944.

One of the flax diseases requiring study under Western Canadian conditions was pasmo. Pasmo affects all aboveground parts of the flax plant, spotting the leaves, blighting flowers and bolls, weakening the pedicels, and causing elongate brown lesions on the stems. The stem lesions enlarge and coalesce to form bands that circle the stem and alternate with unaffected green areas to produce the mottled appearance characteristic of the disease. Diseased plants, which ripen and die prematurely, generally occur in groups, causing brown patches which may enlarge to include the whole field.

The fungus causing pasmo was first described in Argentina in 1911 by Spegazzini as *Phlyctaena* (?) *linicola*, but was renamed *Septoria linicola* (Speg.) Gar. in 1935 (16). The perfect stage, named *Sphaerella linorum* by Wollenweber in 1938, has been found only twice, once by Wollenweber (41) on Argentine material, and once by the writer on fiber flax in Manitoba (11).

Pasmo was found outside South America for the first time in North Dakota in 1916 (5). The disease appeared in Asia, Europe (22), and New Zealand (24) before it was recognized in Canada, where it was found at Ottawa in 1939 and in Manitoba in 1940 (8). Pasmo has occurred in Manitoba each year since 1942 (9, 10, 11, 28, 29, 30, 31, 32) and in Saskatchewan each year since 1946 (38, 39, 40, 32).

The need for investigations of pasmo in Western Canada was indicated by the heavy losses caused by the disease in the North Central States in 1943 (36, 37) and by its severity in other parts of the world (16, 22, 24). This need was emphasized by the apparently greater susceptibility to pasmo of new, rust-resistant varieties of flax that were introduced into Western Canada following an outbreak of rust in 1942.

Many phases of the pasmo problem have been studied since this work was begun in 1944. Some of these studies are still in progress. This paper reports the results of two lines of investigation:

1. Devising effective methods of inoculating flax plants in the greenhouse and of establishing artificial epiphytotics in the field.

2. Determining the effect of pasmo infection on the seed yield and thousand kernel weight of four varieties of flax grown in inoculated plots in the three years 1945 to 1947.

Methods

S. linicola is easily cultured on common laboratory media such as potato dextrose agar. It grows very slowly, the round colonies averaging from 15 to 20 mm. in diameter after 10 days at laboratory temperatures. Spores are exuded in drops from pycnidia which form freely in culture for one or two transfers following isolation. Difficulty was experienced in inducing sporulation in cultures on standard media after several transfers. Sporulation of the fungus on a large selection of media was studied, and was found to be satisfactory on potato dextrose agar with ½ of 1% of yeast extract added.

The first inoculations made by spraying spore suspensions of *S. linicola* in water on young flax plants in the greenhouse at Winnipeg in 1944 resulted in good infection on the cotyledons but practically no infection on the upper leaves. This seemed to corroborate published reports (5, 22, 23) of the resistance to pasmo of flax plants between the cotyledon and blossoming stages. It was observed, however, that although a film of the suspension of spores in water remained on the cotyledons of inoculated plants, droplets of the suspension ran off the upper leaves. As this indicated that surface-tension phenomena might be responsible at least in part for the results, it was decided to add wetting agents to the suspension. The substances tested, all readily available at the time, were gelatin, Aerosol O.T., Nacconal NR, Nacconol NRSF, and Dupont Spreader Sticker. All were effective in wetting flax leaves thoroughly.

The effect of various concentrations of each substance on spore germination and on flax leaves was then studied. In the range of concentrations used, all substances except gelatin inhibited spore germination or injured flax leaves to some extent. Flax leaves were not harmed by gelatin solutions, and spore germination in them was excellent. Concentrations of gelatin of ¼ to 1% in the spore suspension were uniformly satisfactory, and were used in subsequent inoculations. The difference in wetting by water and gelatin suspensions sprayed on to flax seedlings is illustrated in Figs. 1 and 2. Similar improvement of the results of spray inoculations on rice by the use of various wetting and sticking agents, including gelatin, was reported by Andersen and Henry (2).

As pasmo occurred on flax plants dusted and sprayed with various fungicides, the materials readily available could not be used to regulate the intensity of pasmo in the same way that sulphur dust was used to produce various intensities of rust on cereals (20, 3, 4, 17). Various methods and dates of inoculation were therefore tried in 1945 to obtain different degrees of infection. Some plots were inoculated by spreading between the rows flax stems naturally infected with pasmo in 1944. Other plots were sprayed with gelatin suspensions of spores of *S. linicola* when the flax plants were from 3 to 4 in. tall or in bloom. Spray inoculations caused more disease than infected straw spread in the plots, and spraying at different dates produced significantly different results. As it was easier to prepare and standardize spore suspensions than infected flax stems, and, as varying the date of inoculation influenced

the intensity of the resulting infection, spray inoculations made at different dates were used in experiments in subsequent years.

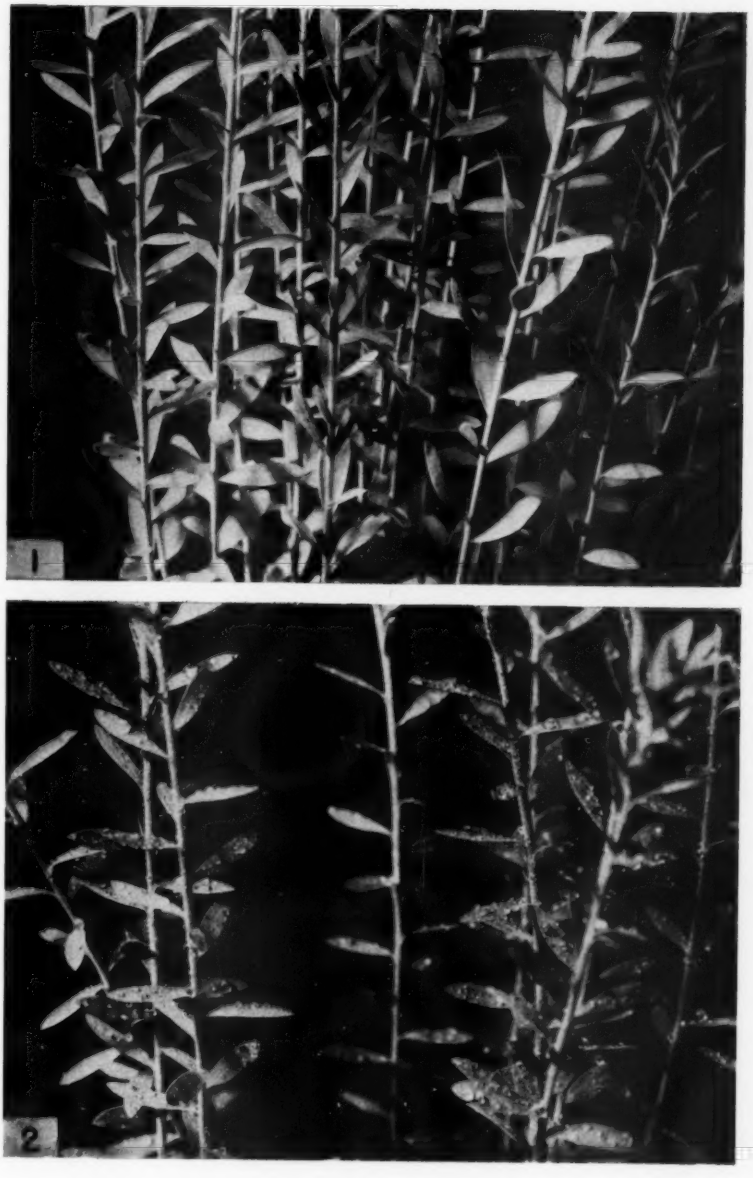
Spores for large-scale inoculation were produced in 250 cc. medicine bottles containing 100 ml. slants of potato dextrose agar to which yeast extract had been added. The bottles withstood repeated sterilization in the autoclave, were easily stored, and were strong enough to be shipped through the mails for use by plant breeders at distant points. The slants were inoculated by spraying with spore suspensions from a de Vilbiss atomizer, aseptic technique being used. The bottles were plugged with cotton and incubated on a laboratory bench until adequate sporulation and freedom from contamination were assured. They were then closed with screw caps and stored in a refrigerator until needed. Very heavy suspensions were obtained by washing spores from each slant with several changes of water, one slant being used to prepare three liters of suspension. The spore suspensions in $\frac{1}{4}$ to 1% gelatin solution were sprayed on full grown flax plants at the rate of one liter to approximately 50 ft. of row, and on plants in the seedling stage at the rate of one liter to approximately 75 ft. of row. Inoculations were made with knapsack sprayers of 2- to 3-gal. capacity, usually in the evening to prevent rapid drying of the spores, such as occurred in the heat of the day.

Four flax varieties, Crystal, Viking, Royal, and Redwing, were grown in suitably replicated plots at Winnipeg and Morden in the three years 1945 to 1947. The new, rust-resistant, yellow-seeded varieties Crystal and Viking were chosen because they were, respectively, among the least susceptible and the most susceptible to pasmo. The brown-seeded varieties Royal and Redwing were included because they were the standard flax varieties throughout much of Western Canada. Certain plots of each variety were inoculated in 1945 by spreading pasmo-infected flax straw between the rows or by spraying the plants with spore suspensions of *S. linicola*, at two stages of plant growth. Spray inoculations were made at five dates in 1946 and four dates in 1947. Disease readings and yield data were obtained at both stations in 1945. The plots at Winnipeg were discarded in 1946 because of poor, uneven germination, and the plots at Morden were discarded in 1947 because a heavy rainstorm several days before harvest stripped most of the bolls from inoculated flax plants. Thus, although plots were seeded at two stations in each of three years, yield and disease data were obtained for only four "station-years". The center two rows of each four-row plot were harvested, dried, and threshed with a Kemp rod-row thresher (19) with the wind shut off so that no seed was lost. The seed was cleaned carefully with sieves and a small fan, to make sure that even the light seed was retained. Thousand-kernel weights were determined for samples from all plots. All data were analysed statistically.

FIG. 1. *Flax seedlings sprayed with suspension of spores of Septoria linicola in water.*

FIG. 2. *Flax seedlings sprayed with suspension of spores of Septoria linicola in water with added gelatin.*

PLATE I



Effects of Inoculations with Pasmo

Disease Data

Good pasmo infections were obtained every year as a result of spraying with heavy spore suspensions. Disease ratings in 1945 were estimates of the percentage of leaves infected. Disease ratings in 1946 and 1947 were estimates of the percentage total stem area lesioned. All statistical analyses involving disease ratings were made according to Bliss's angular transformation of percentage to $\sin^2 \theta$ as given by Snedecor (33) to avoid the bias inherent in percentage figures outside the range 25% to 75%. The transformed mean disease ratings for each variety and each method and date of inoculation, with appropriate measures of significance, are given in Table I.

TABLE I

PASMO DISEASE RATINGS, PERCENTAGES TRANSFORMED TO $\sin^2\theta$, MEANS OF ALL REPLICATES

Inoculation							Minimum significant difference between means		
Stage and method	Days after seeding	Crystal	Viking	Royal	Redwing	Treatment mean		5%	1%
Morden, 1945									
Flowering—spray	64	83	90	86	90	87.26	Variety	3.00	4.11
Seedling—spray	39	74	90	74	84	80.56			
Seedling—straw		51	90	54	68	65.52	Treatment	7.09	11.11
Seedling—chopped straw		54	90	51	57	62.88			
Uninoculated check	96	48	86	42	51	56.69			
Variety mean		60.12	89.32	58.20	67.57				
Winnipeg, 1945									
Flowering—spray	59	74	89	78	80	79.86	Variety	3.70	4.94
Seedling—spray	30	31	66	45	51	48.29			
Seedling—straw		33	64	39	43	44.63	Treatment	7.20	9.97
Seedling—chopped straw		29	55	27	41	37.88			
Uninoculated check	94	12	36	19	22	22.25			
Variety mean		32.33	56.42	38.05	41.72				
Morden, 1946									
Flowering—spray	51	41	90	79	90	74.88	Variety	4.78	6.38
Seedling—spray	36	47	77	63	82	67.20			
Postflowering—spray	58	18	58	35	52	40.62	Treatment	9.85	13.64
Boll filling—spray	65	3	37	3	14	14.40			
Ripening—spray	83	5	58	13	18	23.49			
Uninoculated check	108	0	34	10	14	14.51			
Variety mean		18.97	58.80	33.95	45.05				
Winnipeg, 1947									
Flowering—spray	52	80	90	83	90	85.61	Variety	2.53	3.38
Flowering—spray	58	76	90	85	90	85.29			
Seedling—spray	40	68	90	78	90	81.39	Treatment	3.51	4.87
Flowering—spray (1/100)		68	90	69	90	79.25			
Ripening—spray	74	64	90	60	89	75.74			
Uninoculated check	105	57	90	58	89	73.45			
Variety mean		68.87	90.00	72.10	89.53				

Inoculations made at or about the time the plants were in flower resulted in the highest mean disease ratings in all years at both stations. In every case, inoculations at the most effective dates resulted in significantly higher disease ratings than inoculations at the least effective dates. Seedling inoculations caused less disease than inoculations at flowering, but more than inoculations just before harvest. The effects on disease ratings of spray inoculations at different dates are illustrated in Fig. 3.

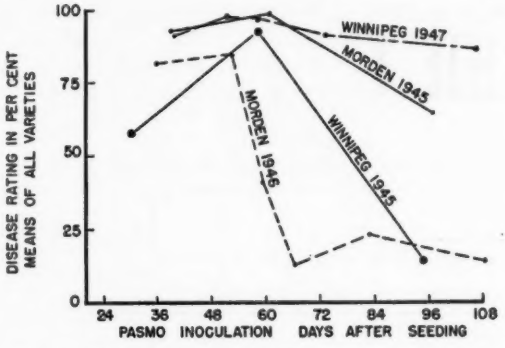


FIG. 3. *Effect of date of inoculation on severity of pasmo infection.*

Uninoculated check plots became infected through spread of the pathogen from other plots, but the disease developed in them relatively late in the season. In 1946 and 1947, disease ratings on the check plots did not differ significantly from those resulting from the least effective inoculations.

The effect of concentration of inoculum on disease intensity was investigated at the third date of inoculation in 1947. One set of plots was sprayed with a heavy suspension, similar to those used in earlier inoculations; another set of plots was sprayed with the same volume of a dilute suspension containing only one one-hundredth as many spores. Spraying with the dilute suspension gave a significantly lower final disease rating than spraying with the heavy suspension, although it was significantly higher than that on uninoculated check plots.

The four varieties studied differed in their susceptibility to pasmo. This was indicated by differences in degree of discoloration as a result of inoculation, and by highly significant differences in disease ratings. Viking was the most susceptible, Redwing was second, Royal was third, and Crystal was the least susceptible. Disease ratings of the four varieties, given as means of all treatments, are illustrated graphically in Fig. 4.

Differences among pasmo infection ratings of the four varieties were highly significant. Analyses of variance proved that the interaction of varieties and inoculations also was highly significant in three station-years and significant at the 5% level in the fourth (Winnipeg, 1945). This finding indicated that

not only did the varieties differ in their susceptibility to pasmo, but that they differed also in their reaction from inoculation to inoculation. The reaction of Crystal at Morden in 1946 is an example of this variation. The

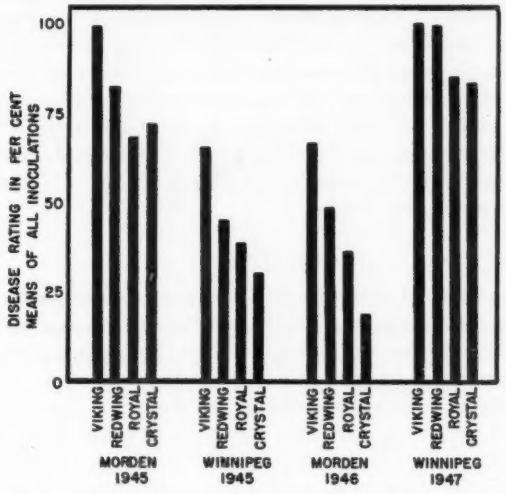


FIG. 4. *Pasmo reaction of four flax varieties in four station-years.*

heaviest disease rating on the three other varieties resulted from the inoculation at flowering (51 days after seeding), but the heaviest disease rating on Crystal resulted from the seedling inoculation (36 days after seeding). The variation in reaction of varieties from inoculation to inoculation is shown graphically in Fig. 5, prepared from the Morden (1946) data. The graph indicates that the varietal response, except that of Crystal, differed in degree, or slope of the curve, rather than in kind, or direction of the curve.

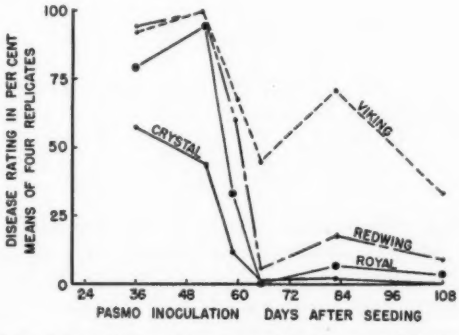


FIG. 5. *Effect of date of inoculation on severity of pasmo infection on four flax varieties at Morden in 1946.*

Rate of ripening was markedly affected by pasmo infection in experimental plots every year. Redwing flax is normally the earliest to ripen of the four varieties included in these experiments. Crystal averages four days longer to maturity and Viking and Royal about six days longer, according to data furnished by plant breeders. Viking, which was consistently the most severely diseased of the four varieties, ripened ahead of Redwing every year, although in uninoculated plots in other experiments it was regularly later than Redwing. Heavily diseased plots of each of the varieties ripened earlier than the less severely diseased plots. In 1945, the plots at Winnipeg were harvested as soon as possible after they matured. Forty-two of 48 severely diseased plots of all varieties were cut early, whereas only 20 of the 48 less diseased plots were ready to harvest at the same date.

Yield Data

Seed yields were affected significantly by inoculation at various intervals after seeding, with some inoculations causing pronounced reductions in yield. The greatest reduction in every case resulted from inoculation at the flowering stage. Mean yields from plots inoculated at flowering were significantly lower than yields from uninoculated check plots and from plots inoculated at the least effective date; they were also significantly lower than yields from plots given the next most effective inoculation in all cases, except in 1945 at Morden, where the difference approached significance. The extent of reductions in yield due to pasmo infection is indicated in Table II, in which

TABLE II

Effect of pasmo infection on yield of flax in inoculated plots

Station	Year	Inoculation (days after seeding)	Yield as percentage of uninoculated check
Morden	1945	39	63
		64	58
Winnipeg	1945	30	63
		59	40
Morden	1946	36	71
		51	55
Winnipeg	1947	52	42
		58	58

mean yields of all plots inoculated at the most effective dates are given as percentages of yield from uninoculated check plots. The figures for yields of individual varieties, expressed as percentage of yield from uninoculated plots of the same variety, are even more striking. Some of the most pronounced reductions are given in Table III. Mean yield data for all tests are given in Table IV.

TABLE III

EFFECT OF PASMO INFECTION ON YIELDS OF INOCULATED PLOTS OF FOUR VARIETIES OF FLAX

Station	Year	Inoculation (days after seeding)	Variety	Yield as percentage of uninoculated check
Winnipeg	1945	59	Viking	30
			Redwing	34
			Royal	40
			Crystal	49
Winnipeg	1947	52	Viking	28
			Royal	37
			Redwing	42
			Crystal	58

TABLE IV

FLAXSEED YIELD IN BUSHELS PER ACRE. MEANS OF ALL REPLICATES

Inoculation							Minimum significant difference between means		
Stage and method	Days after seeding	Crystal	Viking	Royal	Redwing	Treatment mean		5%	1%
Morden, 1945									
Flowering—spray	64	13.68	8.06	16.89	14.75	13.34	Variety	1.552	2.072
Seedling—spray	39	17.62	5.96	19.08	15.31	14.49			
Seedling—straw		17.45	9.18	23.62	17.49	16.94	Treatment	1.720	2.382
Seedling—chopped straw		20.15	10.25	25.25	22.08	19.43			
Uninoculated check	96	24.14	15.31	30.10	23.02	23.14			
Variety mean		18.21	9.42	24.14	18.74				
Winnipeg, 1945									
Flowering—spray	59	18.44	8.73	17.32	10.84	14.18	Variety	1.530	2.042
Seedling—spray	30	23.63	17.18	27.95	20.92	22.42			
Seedling—straw		26.72	18.73	28.47	25.93	24.95	Treatment	2.495	3.455
Seedling—chopped straw		30.10	23.24	32.21	25.09	28.40			
Uninoculated check	94	37.47	29.33	42.91	31.56	35.31			
Variety mean		28.83	20.70	30.98	25.04				
Morden, 1946									
Flowering—spray	51	15.86	10.32	13.92	14.01	13.53	Variety	1.280	1.709
Seedling—spray	36	20.50	14.85	17.34	17.00	17.42			
Postflowering—spray	58	23.23	22.59	22.18	19.94	21.99	Treatment	3.721	5.934
Boll filling—spray	65	23.88	29.39	28.08	23.41	26.19			
Ripening—spray	83	24.02	25.71	26.29	23.34	24.84			
Uninoculated check	108	23.14	28.02	23.24	23.83	24.56			
Variety mean		21.77	21.86	21.88	20.26				
Winnipeg, 1947									
Flowering—spray	52	11.43	4.28	6.82	9.00	7.88	Variety	.980	1.308
Flowering—spray	58	12.91	8.58	8.68	12.92	10.77			
Seedling—spray	40	16.47	6.46	14.31	15.90	13.29	Treatment	1.532	2.121
Flowering—spray (1/100)		18.25	9.93	16.04	19.11	15.83			
Ripening—spray	74	20.66	15.16	18.59	21.46	18.97			
Uninoculated check	105	19.82	15.07	18.64	21.19	18.68			
Variety mean		16.59	9.91	13.33	16.60				

Although the flax plant is reportedly resistant to pasmo between the cotyledon and flowering stages, inoculations of young plants, from 30 to 40 days after seeding, resulted in significantly higher disease ratings and significantly lower yields than inoculations at any other stage except flowering. (The inoculations at 52 and 58 days after seeding at Winnipeg in 1947 came within the flowering period.)

Late inoculations in 1946 and 1947 resulted in slightly higher yields than those obtained from uninoculated check plots. The differences were not statistically significant. It is possible that appreciable leaf infection, which was not estimated in either year, resulted from natural spread of the disease, and that this infection may have been slightly heavier on the check plots or may have affected them earlier, thus accounting for their lower yields. The effect of inoculations at various intervals after seeding on the mean yields from all plots is shown in Fig. 6.

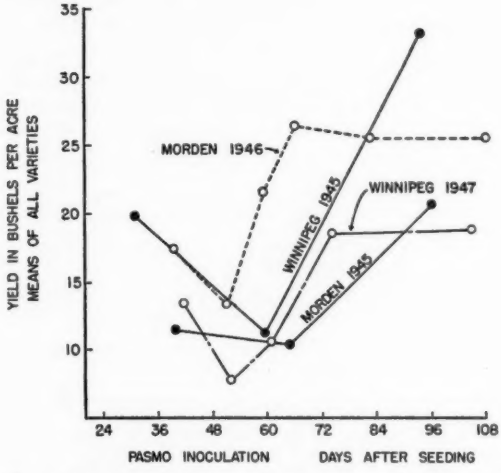


FIG. 6. *Effect of date of inoculation with pasmo on yield of flax in four station-years.*

The relationship between disease and yield was studied by calculating correlation coefficients, given in Table V, from the data for each variety at each station. Transformed values for disease ratings were used. Highly significant negative correlations for individual varieties in all but a few cases indicate that the relationship between disease and yield is very close. Levels required for significance were higher for the Morden, 1945, data, as disease ratings were taken on only half the replicates. The low correlation for Redwing and the zero correlation for Viking at Winnipeg in 1947 were results of the uniformly high disease ratings recorded for all plots of these varieties.

Coefficients for the regression of yield on disease were calculated from the untransformed data for each variety at Morden and Winnipeg in 1945, and

TABLE V

CORRELATION COEFFICIENTS: PASMO DISEASE RATING AND YIELD OF FLAXSEED

Variety	Morden 1945†	Winnipeg 1945	Morden 1946	Winnipeg 1947
Crystal	−.384	−.828**	−.559**	−.478*
Viking	−.709**	−.790**	−.801**	0
Royal	−.949**	−.887**	−.706**	−.692**
Redwing	−.796**	−.877**	−.648**	−.365

† *Disease ratings at Morden, 1945, were taken on only half the replicates.*

* *Significant. Required for significance at 5% point, for Morden, 1945, .576; for all other station-years, .404.*

** *Highly significant. Required for significance at 1% point, for Morden, 1945, .708; for all other station-years, .515.*

at Morden in 1946. The coefficients were used in regression equations to predict yields at given levels of disease. The extremely close agreement between calculated and observed mean values for Royal flax at Morden in 1946 is shown in Fig. 7. The same data were used to calculate the effect of

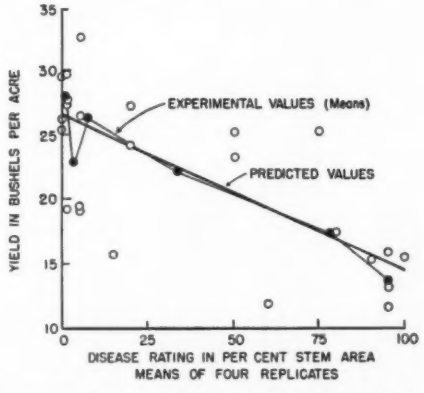


FIG. 7. *Regression of yield on pasmo infection of Royal flax at Morden in 1946.*

various intensities of disease on yield, illustrated in Fig. 8, expressed as percentage of yields expected in the absence of pasmo infection.

Yields of the four varieties differed in each of the station-years. The differences were very highly significant in three of the four station-years, and were significant past the 5% point in the fourth (Morden, 1946). Mean yields of the varieties are presented graphically for each station-year in Fig. 9.

Relative yields of the four varieties differed from station-year to station-year and, as shown by highly significant interactions between inoculation and variety for all data, except those for Winnipeg in 1945, they also differed in

reaction from inoculation to inoculation. Differences in reaction of varieties to inoculations were largely differences in degree, rather than in the kind of effect exerted by different methods and dates of inoculation. This finding is

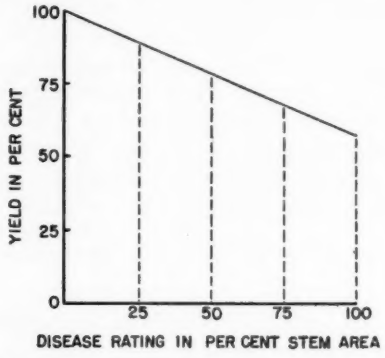


FIG. 8. *Effect of pasmo infection on yield of Royal flax at Morden in 1946, expressed as percentage of yield expected in absence of disease.*

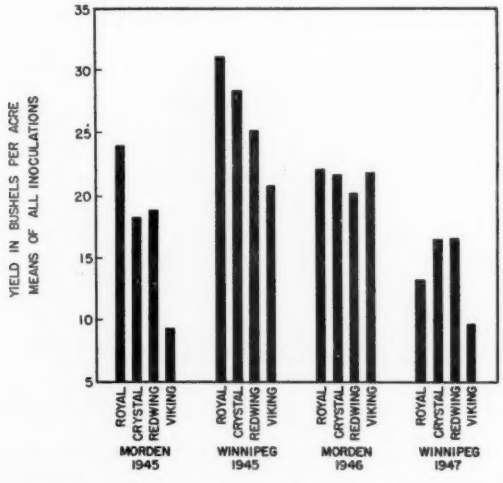


FIG. 9. *Yields of four flax varieties in pasmo-inoculation tests in four station-years.*

illustrated in Fig. 10 by the use of yield data for each of the varieties for each inoculation at Morden in 1946.

Thousand kernel weights were determined as a measure of seed size of samples from all plots in each year. The mean data are presented in Table VI. Statistical analyses of the data showed that differences in thousand kernel

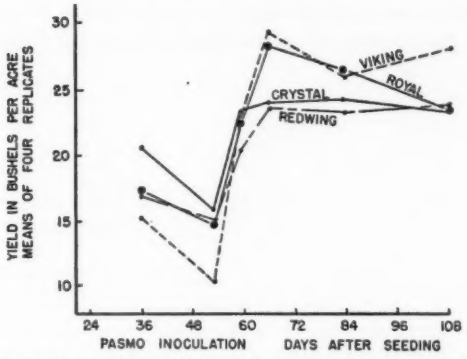


FIG. 10. *Effect of date of inoculation with pasmo on yield of four flax varieties at Morden in 1946.*

TABLE VI

THOUSAND KERNEL WEIGHT OF FLAXSEED IN GRAMS. MEANS OF ALL REPLICATES

Inoculation							Minimum significant difference between means		
Stage and method	Days after seeding	Crystal	Viking	Royal	Redwing	Treatment mean		5%	1%
Morden, 1945									
Flowering—spray	64	5.52	4.55	4.35	3.80	4.55	Variety	.116	.155
Seedling—spray	39	5.67	4.30	4.82	3.95	4.68			
Seedling—straw		6.00	4.80	5.50	4.12	5.10	Treatment	.214	.296
Seedling—chopped straw		6.17	4.85	5.62	4.35	5.25			
Uninoculated check	96	6.52	5.40	5.77	4.67	5.60			
Variety mean		6.02	4.79	5.29	4.23				
Winnipeg, 1945									
Flowering—spray	59	4.72	4.17	3.95	3.35	4.05	Variety	.172	.230
Seedling—spray	30	5.40	4.30	4.38	3.92	4.48			
Seedling—straw		5.55	4.60	4.70	4.00	4.71	Treatment	.280	.388
Seedling—chopped straw		5.90	5.10	5.00	4.35	5.09			
Uninoculated check	94	6.15	5.32	5.32	4.57	5.34			
Variety mean		5.62	4.75	4.79	4.06				
Morden, 1946									
Flowering—spray	51	5.35	4.79	4.96	4.11	4.86	Variety	.086	.116
Seedling—spray	36	5.37	5.07	5.15	4.34	5.04			
Postflowering—spray	58	5.57	5.34	5.21	4.68	5.23	Treatment	.099	.138
Boll filling—spray	65	5.42	6.01	5.44	4.58	5.42			
Ripening—spray	83	5.57	6.01	5.51	4.75	5.52			
Uninoculated check	108	5.64	6.05	5.33	4.62	5.46			
Variety mean		5.49	5.55	5.27	4.46				
Winnipeg, 1947									
Flowering—spray	52	4.32	4.63	3.88	3.53	4.21	Variety	.124	.166
Flowering—spray	58	4.53	4.37	3.75	3.59	4.06			
Seedling—spray	40	5.08	4.56	4.34	3.75	4.43	Treatment	.244	.338
Flowering—spray (1/100)		4.96	4.49	4.17	4.04	4.41			
Ripening—spray	74	5.34	5.01	4.56	4.41	4.83			
Uninoculated check	105	5.30	5.34	4.68	4.42	4.93			
Variety mean		5.00	4.73	4.23	3.95				

weights due to different inoculations were highly significant in all cases. The effect of inoculation at different dates on mean weights per thousand kernels is shown by graphs in Fig. 11.

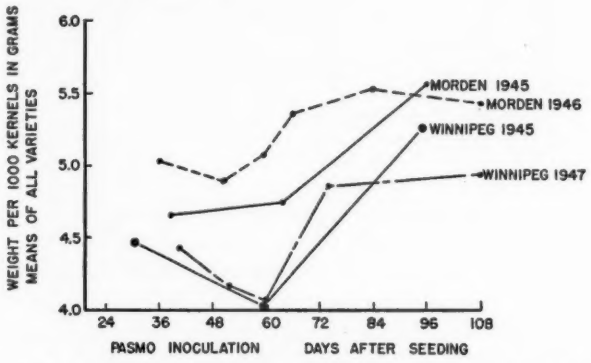


FIG. 11. *Effect of date of inoculation with pasmo on weight per 1000 kernels of flax in four station-years.*

Seed size of the respective varieties was affected differently by various dates of inoculation, as shown by highly significant interactions between inoculation and variety in three station-years, and an interaction approaching significance in the fourth, at Winnipeg, 1947. The interaction is illustrated by data from Morden, 1946, presented in Fig. 12.

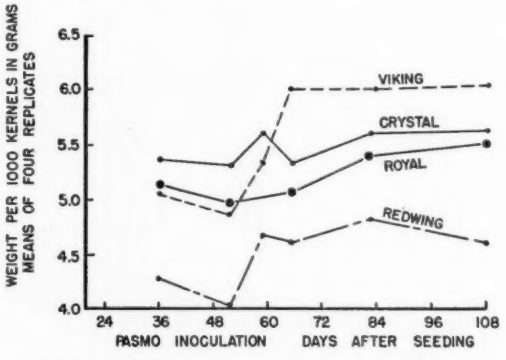


FIG. 12. *Effect of date of inoculation with pasmo on weight per 1000 kernels of four varieties of flax at Morden in 1946.*

The relationship between intensity of disease and seed size was determined by calculating correlation coefficients, given in Table VII, for these data for each variety in each year. Transformed values were used for the disease ratings. The highly significant negative correlations show that, in most

TABLE VII

CORRELATION COEFFICIENTS: PASMO DISEASE RATING AND THOUSAND KERNEL WEIGHT OF FLAXSEED

Variety	Morden 1945†	Winnipeg 1945	Morden 1946	Winnipeg 1947
Crystal	−.630*	−.867**	−.405*	−.293
Viking	−.609*	−.719**	−.816**	0
Royal	−.931**	−.890**	−.661**	−.554**
Redwing	−.820**	−.797**	−.777**	−.278

† *Disease ratings at Morden, 1945, were taken on only half the replicates.*

* *Significant. Required for significance at 5% point, for Morden, 1945, .576; for all other station-years, .404.*

** *Highly significant. Required for significance at 1% point, for Morden, 1945, .708; for all other station-years, .515.*

cases, the heavier the pasmo infection, the greater was the reduction in seed size. The low correlation for Redwing and zero correlation for Viking at Winnipeg in 1947 are results of the uniformly high disease ratings on all plots of these varieties.

Coefficients were calculated for the correlation between thousand kernel weight and yield data. They are given in Table VIII. The highly significant positive correlations for all data, except those for Crystal at Morden

TABLE VIII

CORRELATION COEFFICIENTS: THOUSAND KERNEL WEIGHT AND YIELD OF FLAXSEED

Variety	Morden 1945	Winnipeg 1945	Morden 1946	Winnipeg 1947
Crystal	.643**	.867**	.401†	.648**
Viking	.876**	.720**	.898**	.697**
Royal	.884**	.881**	.903**	.798**
Redwing	.797**	.696**	.721**	.888**

** *Highly significant. Required for significance at 1% point, .515.*

† *Required for significance at 5% point, .404.*

in 1946, indicate strongly that much of the loss in yield of the various plots was caused by a reduction in the size of individual seeds. This result agrees with the finding of Newton, Peturson, and Meredith (25) that leaf rust caused significant reductions in yield and kernel weight of five out of the six varieties of barley they tested, and with the results of Peturson and Newton (26) that leaf rust of wheat reduced yields by reducing the size of individual kernels.

Reduction in seed size is not, however, the only means by which seed yields may be seriously decreased. Pasmo infection makes flax pedicels brittle. Heavy rains or strong winds just before harvest may break off a

high proportion of the bolls from diseased pedicels. Such breaking occurred in the plots at Morden in 1947, when 3 in. of rain fell in a few hours several days before the plots were to be harvested, and stripped almost all the bolls from diseased plants. Similar damage was observed in several farm fields in 1947, causing about 50% loss in one field. Fig. 13 shows stripped pedicels, and Fig. 14 shows bolls lying on the ground. Both photographs were taken at Morden in 1947.

Discussion

Methods were devised for inoculating flax plants with pasmo in the greenhouse and for establishing artificial epiphytotics of pasmo in the field. The need for such methods was indicated by the irregular, patchy occurrence of natural pasmo infections, which made it difficult or impossible for pathologists and flax breeders to assess varietal reaction reliably in uninoculated plots. Simple, effective methods of inoculation were also required in order to proceed with the studies contemplated, some of which are reported in this paper.

The need for plant pathologists to pay attention to crop loss measurements in comprehensive studies of individual diseases was pointed out by Chester (7). As a result of questions directed to plant pathologists on the estimation of crop loss caused by definite intensities of various diseases, he concluded that ". . . . we are faced with an obligation to develop this neglected phase of phytopathology . . .". Tervet (35) pointed out that losses from the outbreak of pasmo in the United States in 1943 were unquestionably heavy, but that exact figures of the effect of the disease on yield were difficult to obtain. Estimates of losses caused by pasmo in various parts of the world have been published (27, 37, 6, 15, 1, 16, 22, 24), but none of them was obtained from controlled experiments. Stone (34), working with potatoes, pointed out the difficulty of evaluating disease effects without using an experimental basis, particularly in seasons when even diseased plots may produce yields as high as or higher than the average for the area.

An approach to the problem of determining the effect of disease on yield of field crops was indicated by Goulden and Greaney (17). They proved statistically that the regression of yield on stem rust of wheat was linear: uniform increases in rust intensity, whether infection was light or heavy, resulted in uniform reductions in yield. In two of their experiments, each 10% increase in rust infection resulted in 6.8% and 9.7% reduction in yield. A similar conclusion might be based on the data for yield and pasmo infection of Royal flax in the 1945 and 1946 experiments. Each 25% increase in pasmo infection reduced yields by 14% and 10.7%, respectively. It is not advisable, however, to generalize about the effect of pasmo on yield. Correlations between

FIG. 13. *Pedicels of pasmo-infected flax plants stripped by heavy rain.*

FIG. 14. *Bolls stripped from pasmo-infected flax plants by heavy rain.*

13

14

disease and yield, although highly significant in most cases, were not significant for all varieties in all years. Low correlation was attributable to uniformly high infection in all plots, including checks, of two of the varieties in one experiment. Even though definite variations in yield resulted from inoculation at various dates, the disease developed so rapidly that differences in infection were no longer apparent at the time disease ratings were made. Pasmo infection, unlike rust infection, continues to spread on individual ripe plants. Late infections, which might do relatively little harm, might possibly appear as severe as infections which developed earlier and inflicted considerable injury on the plants. It is important, therefore, to qualify all estimates of loss caused by pasmo, according to the time of infection. Heavy infection at or about the time of flowering might reduce the yield of a moderately susceptible variety by 20% to 30%, and of an extremely susceptible variety by 50% or more. It is important also to determine the time of infection, not as the time at which conspicuous lesions appear on the stems, but as the time at which numerous spots appear on the leaves.

Leaf disease ratings were used as a measure of disease intensity in 1945, and stem disease ratings in 1946 and 1947. The extremely high correlations between yields and disease ratings based on leaf and on stem infections in 1945 and 1946, respectively, indicate that the two types of disease data are very closely comparable, and that both are valid for the purpose for which they were used in the experiments reported.

Klages (21) found definite yield reductions from mechanical defoliation of flax at various stages of growth, from seedlings 6 in. high to plants in the hard-dough stage. Defoliation of plants from flower bud formation to flowering caused the greatest reduction in yield (38%), less at the hard- and soft-dough stages (32% and 29% respectively) and at full bloom (27.8%), and considerably less during the early stages (19%), when plants were able to produce new leaves rapidly. These results differ from those obtained by inoculating flax plants at various stages with pasmo: inoculation at flowering was much more injurious than at the soft- and hard-dough stages, and seedling inoculations also reduced yields much more than late-season inoculations. Klages pointed out that mechanical defoliation of flax plants in the seedling stage reduced yields relatively little, because young plants were able to produce new leaves quickly, whereas older plants did not recover as readily. Defoliation as a result of pasmo infection apparently affects plant development differently than does mechanical defoliation. Seedlings defoliated by the disease are set back in growth, and do not produce basal branches as readily as do healthy plants. Differences between the effects of defoliation and of pasmo inoculation at later stages in plant development also indicate that damage by pasmo is the result of more factors than defoliation (reduction in photosynthetic area) alone.

Dillman (12) made careful determinations of the growth of flax seeds at daily intervals from the time the plants flowered until they matured. He found that seeds attained their maximum volume in from 12 to 14 days after

the plants had flowered, but decreased slightly in size during the last few days of the ripening period. Dry weight of the seeds increased uniformly to a maximum at 33 days, several days before the normal harvesting stage, from 39 to 40 days after the plants had come into flower. Dillman and Hopper (13) made an exhaustive study of the effect of climate on the seed yield and seed size of four varieties of flax. They found that deficient precipitation during the growing season, and high temperatures in July, shortened the vegetative and fruiting periods. Enforced early ripening reduced yields and lowered weight per thousand kernels. Flor (14) found that natural infection by flax rust also reduced seed yield and seed size of flax.

Greaney, Woodward, and Whiteside (18) reported that stem rust of wheat disturbed normal plant metabolism, arrested plant development, and induced early ripening. Enforced early ripening checked movement of food materials to the seed, interfering considerably more with the deposition of organic than inorganic compounds in the kernels. As a result, much elaborated food remained in the straw and the grain was immature, small, shrivelled, and low in protein.

Flax was affected by pasmo inoculation in much the same way as by adverse weather conditions and by rust, and in much the same way as wheat is affected by stem rust. Plant development was arrested. Heavily diseased plants were shorter than healthy plants, branched less, and ripened more rapidly. As a result (although no analyses were made to ascertain whether organic constituents remained in the straw), there was less deposition in the seeds, which were immature, small, and shrunken.

Acknowledgments

The author derives much pleasure from recording his gratitude to: Dr. J. H. Craigie, Dominion Botanist, who suggested the investigation; Dr. W. F. Hanna, Officer-in-Charge, Dominion Laboratory of Plant Pathology, Winnipeg, who gave encouragement in the course of the work and read the manuscript critically; Dr. C. H. Goulden, Dominion Cerealist, who gave advice on the statistical design of the experiments and on methods of analyzing the data, and Prof. G. B. Oakland, Department of Actuarial Science, University of Manitoba, who advised on the use of angular transformations in analyzing the percentage data; and Dr. E. C. Stakman and Dr. J. J. Christensen, Division of Plant Pathology, University of Minnesota, St. Paul, who read the manuscript.

References

1. ALTSTATT, G. E. Flax diseases in Texas. Plant Disease Reptr. 28 : 256. 1944.
2. ANDERSEN, A. L. and HENRY, B. W. The use of wetting and adhesive agents to increase the effectiveness of conidial suspensions for plant inoculations. Phytopathology, 26 : 1056-1057. 1946.
3. BAILEY, D. L. and GREANEY, F. J. Preliminary experiments on the control of leaf and stem rusts of wheat by sulphur dust. Sci. Agr. 6 : 113-117. 1925.
4. BAILEY, D. L. and GREANEY, F. J. Dusting with sulphur for the control of leaf and stem rust of wheat in Manitoba. Sci. Agr. 8 : 409-432. 1928.

5. BRENTZEL, W. E. The pasmo disease of flax. J. Agr. Research, 32 : 25-37. 1926.
6. BRETZ, T. W. Summary of plant diseases observed in Iowa during 1943. Plant Disease Reptr. Suppl. 147 : 217-228. 1944.
7. CHESTER, K. STARR. How accurately are we estimating plant disease losses? Plant Disease Reptr. 29 : 716-718. 1945.
8. CONNERS, I. L. Twentieth annual report of the Canadian Plant Disease Survey, 1940. 1941.
9. CONNERS, I. L. and SAVILE, D. B. O. Twenty-second annual report of the Canadian Plant Disease Survey, 1942. 1943.
10. CONNERS, I. L. and SAVILE, D. B. O. Twenty-third annual report of the Canadian Plant Disease Survey, 1943. 1944.
11. CONNERS, I. L. and SAVILE, D. B. O. Twenty-fourth annual report of the Canadian Plant Disease Survey, 1944. 1945.
12. DILLMAN, A. C. Daily growth and oil content of flaxseeds. J. Agr. Research, 37 : 357-377. 1928.
13. DILLMAN, A. C. and HOPPER, T. H. Effect of climate on the yield and oil content of flaxseed and on the iodine number of linseed oil. U.S. Dept. Agr. Tech. Bull. 844. 1943.
14. FLOR, H. H. Relation of rust damage in seed flax to seed size, oil content, and iodine value of oil. Phytopathology, 34 : 348-349. 1944.
15. FLOR, H. H. Comparative tolerance to pasmo in some new hybrid selections. Proc. Flax Institute of U.S.A., 1944. Mimeographed. 1944.
16. GARASSINI, L. A. El "pasmo" del lino *Phlyctaena* (?) *linicola* Speg. Ensayo a campo de resistencia varietal y estudio morfologico y fisiologico del parasito. Rev. facultad agron., La Plata, 20 : 170-261. 1935.
17. GOULDEN, C. H. and GREANEY, F. J. The relation between stem rust infection and the yield of wheat. Sci. Agr. 10 : 405-410. 1930.
18. GREANEY, F. J., WOODWARD, J. C., and WHITESIDE, A. G. O. The effect of stem rust on the yield, quality, chemical composition, and milling and baking properties of Marquis wheat. Sci. Agr. 22 : 40-60. 1941.
19. KEMP, H. J. Mechanical aids to crop experiments. Sci. Agr. 15 : 488-506. 1935.
20. KIGHTLINGER, C. V. Preliminary studies on the control of cereal rusts by dusting. Phytopathology, 15 : 611-613. 1925.
21. KLAGES, K. H. W. The effects of simulated hail injury on flax. J. Am. Soc. Agron. 25 : 534-540. 1933.
22. KRÜGER, E. Untersuchungen über zwei der bedeutendsten Leinparasiten—*Colletotrichum lini* Manns et Bolley und *Septoria linicola* (Speg.) Gar. (*Sphaerella linorum* Wr.). Arb. biol. Reichsanstalt Land-u.Forstw., Berlin-Dahlem, 23 (2) : 163-188. 1941.
23. LOUGHNANE, J. B., MCKAY, R., and LAFFERTY, H. A. Observations on the pasmo disease of flax and on the causal fungus *Sphaerella linorum* Wollenweber. Sci. Proc. Roy. Dublin Soc. 24 (10) : 89-98. 1946.
24. NEWHOOK, F. J. Pasmo (*Sphaerella linorum*) on flax in New Zealand. New Zealand J. Sci. Technol. 24 (2A) : 102A-106A. 1942.
25. NEWTON, M., PETURSON, B., and MEREDITH, W. O. S. The effect of leaf rust of barley on the yield and quality of barley varieties. Can. J. Research, C, 23 : 212-218. 1945.
26. PETURSON, B. and NEWTON, M. The effect of leaf rust on the yield and quality of Thatcher and Renown wheat in 1938. Can. J. Research, C, 17 : 380-387. 1939.
27. RODENHISER, H. A. Physiologic specialization and mutation in *Phlyctaena linicola* Speg. Phytopathology, 20 : 931-942. 1930.
28. SACKSTON, W. E. Flax diseases in Manitoba in 1945. Can. Plant Disease Survey, 1945. 25 : 32-35. 1946.
29. SACKSTON, W. E. Flax diseases in Manitoba in 1946. Can. Plant Disease Survey, 1946. 26 : 25-26. 1947.
30. SACKSTON, W. E. Flax diseases in Manitoba in 1947. Can. Plant Disease Survey, 1947. 27 : 30-32. 1948.
31. SACKSTON, W. E. Flax disease surveys of the three prairie provinces in 1948. Can. Plant Disease Survey, 1948. 28 : 24-28. 1949.
32. SACKSTON, W. E. Flax diseases in Manitoba in 1949. Can. Plant Disease Survey, 1949. 29 : 28-30. 1950.

33. SNEDECOR, G. W. Statistical methods. 4th ed. Iowa State College Press, Ames, Iowa. 1946.
34. STONE, G. M. Late blight in Southern Alabama. Plant Disease Reptr. 29 : 444-445. 1945.
35. TERVET, I. W. Plant disease survey in Minnesota. Plant Disease Reptr. Suppl. 147 : 210-217. 1944.
36. TERVET, I. W. Diseases in North Dakota in 1943. Plant Disease Reptr. Suppl. 149 : 305-308. 1944.
37. TERVET, I. W. Diseases in South Dakota in 1943. Plant Disease Reptr. Suppl. 149 : 309-311. 1944.
38. VANTERPOOL, T. C. Flax diseases in Saskatchewan in 1946. Can. Plant Disease Survey, 1946. 26 : 23-25. 1947.
39. VANTERPOOL, T. C. Flax diseases in Saskatchewan in 1947. Can. Plant Disease Survey, 1947. 27 : 28-30. 1948.
40. VANTERPOOL, T. C. Flax diseases in Saskatchewan in 1948. Can. Plant Disease Survey, 1948. 28 : 22-24. 1949.
41. WOLLENWEBER, H. W. "*Sphaerella linicola*" N. sp. Die Ursache der Amerikanischer Leinpest. (Pasmo—oder "Septoria"—Krankheit.). Lilloa, Rev. Botan. Inst. 2 : 483-495. 1938.
42. WOOD, H. E. Report on crops, livestock, etc. produced in Manitoba in 1940. Man. Dept. Agr. and Immigration. Crop Bull. 119. 1941.
43. WOOD, H. E. Report on crops, livestock, etc. produced in Manitoba in 1949. Man. Dept. Agr. and Immigration. Crop Bull. 128. 1950.

STORAGE OF ISOLATED CHLOROPLASTS WITHOUT LOSS OF PHOTOCHEMICAL ACTIVITY[1]

By P. R. Gorham[2] and K. A. Clendenning[2]

Abstract

Fragmented spinach chloroplasts were stored for one year in 0.5 *M* sucrose at −40° C. with almost negligible losses of photochemical activity. Thawed chloroplasts and freshly isolated chloroplasts showed similar rates of deterioration at 10° C. Washed chloroplasts did not show as high retention of activity during low temperature storage as crude chloroplast suspensions. High retentions of activity were observed with chloroplasts from different plant species, as well as with different oxidants. The activities of subsamples that were frozen and thawed in a standard way agreed within 5%. Deterioration during lyophilization of chloroplasts occurred chiefly during the early stages of drying, and was lessened by sucrose. The stability of lyophilized chloroplasts in storage varied directly with the degree of desiccation and inversely with the storage temperature; little deterioration occurred in thoroughly dried chloroplasts during lengthy storage at or below 5° C.

Introduction

Isolated spinach chloroplasts were recently stored for the first time at −40° C. with negligible losses of photochemical activity (3). The success of these preliminary experiments prompted the present detailed investigation of chloroplast storage at low temperatures.

Materials and Methods

Chloroplast Sources

Spinach, *Spinacia oleracea* L. var. Long Standing Bloomsdale, was used throughout as the principal source of chloroplasts. One lot of commercial spinach from California was used for experiments on sucrose substitutes and another lot from Texas for experiments on reproducibility. Other species used included New Zealand spinach, *Tetragonia expansa* Thunb.; wheat, *Triticum vulgare* Vill. var. Coronation; millet, *Panicum miliaceum* L. var. Crown; Swiss chard, *Beta vulgaris* L. var. Giant Lucullus; and flax, *Linum usitatissimum* L. var. Red Wing. Except as noted, all plants were grown in the greenhouse under conditions already described (2).

Chloroplast Suspensions

Separated chloroplasts were prepared with the Waring blendor by the method used by Holt and French (7) and MacDowell (10): 25 gm. laminae were ground at full speed for three minutes in 140 ml. 0.5 *M* sucrose; the brei was then filtered through parachute nylon. A centrifugal force of 1800 g. rather than 800 g. (7, 10) was required to separate the chloroplasts satisfactorily. "Washed chloroplasts" were resuspended to the original volume with 0.5 *M* sucrose and centrifuged again before being resuspended for use.

[1] *Manuscript received July 21, 1950.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Issued as N.R.C. No. 2203.

[2] *Biochemist, Plant Science Investigations.*

Chloroplasts prepared in this manner were almost entirely intact. "Grana" or fragmented chloroplasts were prepared by grinding the laminae in sand without added liquid. These were separated from the cell sap by centrifuging for 15 min. at 15,000 g. and then suspended in distilled water to half the initial volume. "Washed grana" were resuspended to the original volume in water and centrifuged again at 15,000 g. before being resuspended for use.

Freezing and Thawing

It is good practice to freeze the chloroplast suspensions rapidly with dry ice before storing them at −40° C. Three- to five-milliliter aliquots of chloroplasts in 0.5 *M* sucrose were usually frozen in slanted 20 ml. plastic tubes between layers of powdered dry ice. Larger lots (up to 300 ml.) were frozen in shallow pans, removed and ground in a meat chopper at −40° C., bottled, and stored at the same temperature. The preparatory "snap-freezing" is not a critical factor; complete retention of photochemical activity has been obtained with the slow freezing that occurs when the suspensions are stored directly at an air temperature of −40° C., as well as with instantaneous freezing in liquid air. The necessity of storing the frozen chloroplasts at or below −40° C., as well as the beneficial effects of 0.5 *M* sucrose, have been reported previously (3).

The following method of thawing the chloroplasts has proved uniformly satisfactory: 3-5 gm. of frozen chloroplast suspension in a plastic tube is continuously stirred with a stout wire at room temperature until a small thermometer can be inserted in the slush; rapid stirring is continued until the sample temperature reaches −0.5° to 0° C. The suspension is then placed in an ice water bath.

Lyophilization

(*a*) *Manifold method.*—Three-milliliter aliquots of chloroplast suspension were placed in chilled ampoules and frozen as a thin shell by rotating the ampoules in dry ice. The ampoules were immediately attached to a vacuum manifold and lyophilized. Pressures of 20 μ or less were maintained throughout the two- or three-hour drying period. The samples were then sealed under vacuum and stored in darkness at −40° to + 70° F. (−40° to 21° C.).

(*b*) *Desiccator method.*—Fifty-milliliter aliquots of chloroplast suspension were frozen in large Petri dishes with dry ice and transferred at once to a large vacuum desiccator. Three or four dishes were stacked in the desiccator with half-inch air spaces between them, the bottom dish resting on a cork ring. Vacuum was applied immediately, and drying at 1 mm. pressure was continued for 6–24 hr. The dry product was powdered, weighed, bottled, and stored as above.

With either method of freeze-drying, reconstituted suspensions, microscopically indistinguishable from fresh preparations, were obtained by stirring the chilled powder in the original volume of ice-water until lump-free.

Activity Measurements

The methods used in estimating photochemical activity (chiefly the Holt and French titration method at 10.0° C.) have been described elsewhere (2, 6). The light intensity used in the titration method was in excess of that required to saturate both moderately active and highly active thawed chloroplasts (Fig. 1). The activities were expressed in terms of total chlorophyll content, which was determined colorimetrically (2).

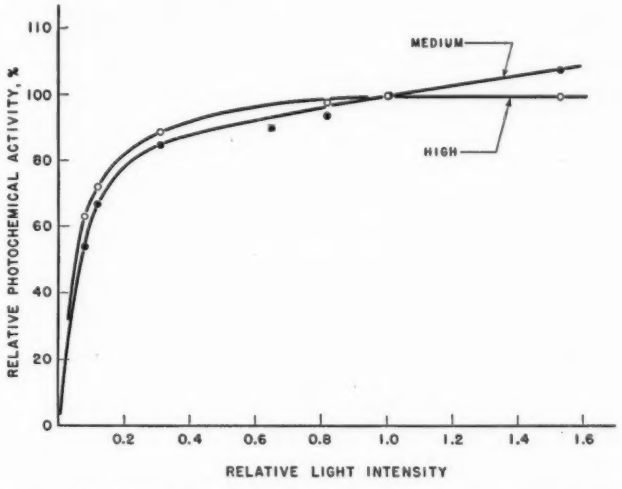


FIG 1 *Light saturation curves for the Hill reaction, using crude spinach chloroplast suspensions that were thawed after low temperature storage. Intensity of 3200 ft-c. before orange filter, as normally employed = 1.0. Q_H^{Fe} (normal light intensity): "medium activity" sample 760, "high activity" sample 1250. Chlorophyll content: 0.60 mgm. per vessel in both samples.*

Results

Reproducibility of Method

Lengthy experiments on isolated chloroplasts might be improved through the use of a single lot of frozen chloroplasts, from which subsamples could be taken for a matter of days or weeks and thawed immediately before use. To be useful in such applications, the photochemical activities observed in the successively thawed aliquots should show less variation than between different preparations of fresh chloroplasts, isolated successively from a common stock of leaves. The stability after thawing also should compare favorably with that of freshly isolated chloroplasts.

The variability of thawed chloroplast and grana suspensions (Table I) refers to aliquots of a single suspension that were frozen, stored, thawed, and tested separately. The variability of the unfrozen chloroplasts and grana refers to freshly prepared suspensions that had been isolated separately from the same lot of spinach leaves.

TABLE I

REPRODUCIBILITY OF SPINACH CHLOROPLAST ACTIVITY MEASUREMENTS *within* GRINDS, USING FROZEN PREPARATIONS, AND *between* GRINDS, USING RANDOMIZED SAMPLES FROM A SINGLE LEAF POPULATION

Chloroplast suspension	Chlorophyll content, mgm. per ml.	No. of replicates	Photochemical activity, $Q_{H^+}^{Ch}$	Coefficient of variation, %
Crude grana, frozen	0.59	9	760 ± 20	3
Crude grana, frozen	0.51	9	760 ± 20	3
Crude whole chloroplasts, frozen	0.56	12	1340 ± 70	5
Crude grana	0.48	8	1090 ± 110	10
Crude grana	0.48	8	1200 ± 60	5
Crude grana	0.48	8	1170 ± 140	12
Separated whole chloroplasts	0.70	8	1070 ± 80	8

The following precautions were taken to reduce the variability of the separately prepared suspensions to a minimum: A large sample of mature, healthy leaves was selected from a bushel of Texas spinach. Each leaf was cut into proximal, middle, and distal portions of about equal area; each of the three piles of leaf parts was mixed thoroughly and stored at 1° C. Four leaf pieces drawn at random from each of the three piles comprised a sample (24 ± 3 gm.). Eight separately prepared crude grana suspensions were tested daily for three days, and eight "separated chloroplast" suspensions were tested on the fourth.

The special effort to obtain uniform leaf samples reduced the variability between "grinds" to about 10%. The variation between successively thawed lots of frozen chloroplast suspension was 4 ± 1% (Table I). The variability of activity measurements on thawed aliquots was no larger than the variability of activity measurements made on unfrozen aliquots.

Stability of Thawed Chloroplast Suspensions

Fig. 2 shows that the rates of deterioration at 10° C. are similar in freshly isolated chloroplasts and in chloroplasts thawed after storage at −40° C. Addition of 10% propylene glycol (or 15% methanol) (4, 8, 11) appeared to stabilize the activity for approximately two hours at 10° C., but resulted in negligible improvement with longer storage periods at this temperature.

Stability During Lengthy Storage at −40° C.

Fig. 3 shows the residual photochemical activities observed in crude spinach chloroplasts and grana during approximately one year's storage at −40°C.

All subsamples withdrawn from the four lots of frozen chloroplasts and grana showed high photochemical activity. The variations in activity ranged from 0 – 28%. The chlorophyll content remained constant. The different

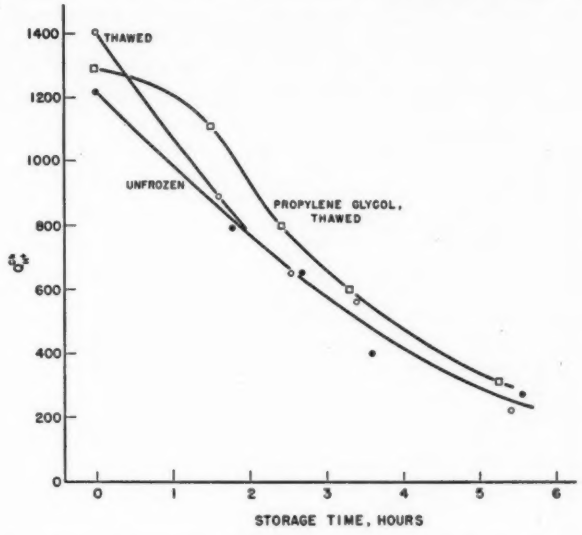


FIG 2 *Storage deterioration of photochemical activity at 10° C. Chlorophyll content: 0.5 mgm. per vessel.*

● *freshly prepared crude grana in 0.5 M sucrose;*
○ *crude grana frozen and thawed in 0.5 M sucrose;*
□ *same but in presence of sucrose + 10% propylene glycol.*

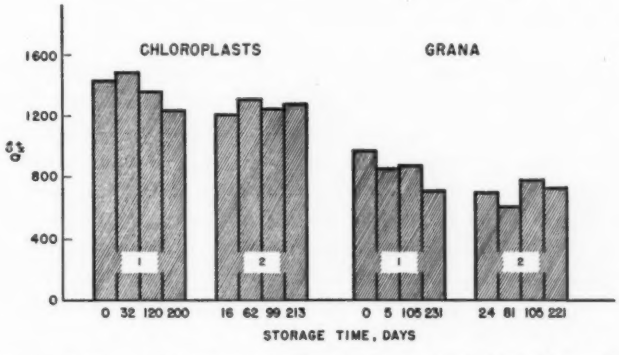


FIG. 3. *Retention of photochemical activity in crude spinach chloroplasts and grana during prolonged storage at −40° C. Chlorophyll contents: chloroplasts (1) 0.56 mgm., (2) 0.60 mgm.; grana (1) 0.59 mgm., (2) 0.51 mgm. per vessel.*

Q_H^{ch} after approximately one year's storage (343-374 days): chloroplasts (1) 1310, (2) 1250; grana (1) 900, (2) 840.

levels of photochemical activity in the chloroplasts and grana (Fig. 3) were caused by the use of different lots of spinach; the photochemical activities of intact chloroplasts and of chloroplast fragments from a common source usually do not differ significantly.

Fig. 4 reports the deterioration in photochemical activity that occurred when washed spinach chloroplasts were stored for two months at −40° C. in 0.5 *M* sucrose. Activity measurements were made both in the presence and

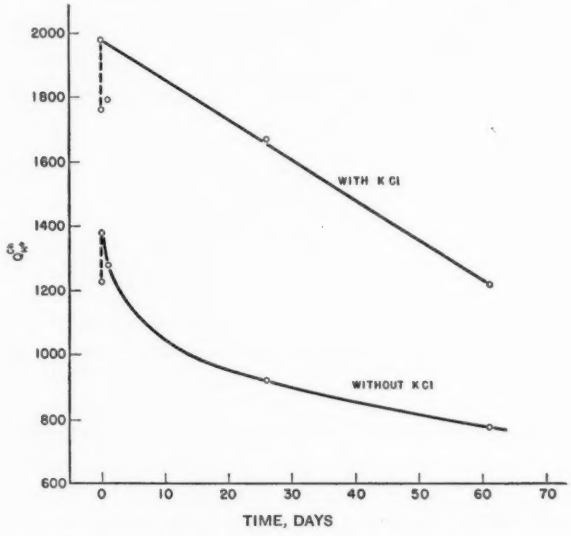


FIG. 4. *Changes in photochemical activity of washed spinach chloroplasts associated with snap-freezing and storage at −40° C. Chlorophyll content: 0.80 mgm. per vessel.*

absence of 0.01 *M* potassium chloride (1). The initial increase in activity resulting from freezing is judged significant since it has been observed repeatedly with isolated whole chloroplasts. Potassium chloride did not abolish this "freezing increment" (Fig. 4). Following the initial rise, there was a continuous decline in activity during storage, whether the tests were conducted in the presence or absence of 0.01 *M* potassium chloride. The presence of sucrose in the frozen suspensions and of potassium chloride in the reaction medium thus did not completely stabilize intact, washed spinach chloroplasts.* Despite the progressive decline in activity, the washed chloroplasts remained highly active after two months' storage ($Q_{H^+}^{ch} = 1220$).

The data of Table II refer to intact spinach chloroplasts that were washed twice, prior to freezing and subsequent testing in the presence of 0.01 *M*

* *When frozen in the presence of 0.11 M potassium chloride and 0.5 M sucrose, storage deterioration was even more rapid than in the presence of sucrose.*

TABLE II

EFFECTS OF SUCROSE ON THE RETENTION OF PHOTOCHEMICAL ACTIVITY IN WASHED SPINACH CHLOROPLASTS DURING STORAGE AT −75° C.

(Activities were measured in the presence of 0.01 *M* potassium chloride, chlorophyll content 0.23 mgm. per vessel)

Storage time, hr.	$Q_{H^+}^{ch}$ before freezing	$Q_{H^+}^{ch}$ after freezing		
		Sucrose molarity		
		0	0.1	1.0
0–4	2260	1340	2160	1770
48		1450	1820	1720

potassium chloride. There was approximately 60% retention of activity in the absence of sucrose and approximately 80% retention of activity when the chloroplasts were frozen and thawed in the presence of 0.1 − 1.0 *M* sucrose.

The deterioration in activity that occurred when crude grana suspensions from five species other than spinach were stored at −40° C. in the presence of 0 − 1.0 *M* sucrose is shown in Table III. The photochemical activity of the grana suspensions from all five species remained high after two to three

TABLE III

PHOTOCHEMICAL ACTIVITY OF CRUDE GRANA OF DIFFERENT SPECIES, SNAP-FROZEN AT −75° C. AND STORED AT −40° C.

(All tests in the presence of 0.01 *M* added potassium chloride)

Species	Chlorophyll, mgm. per ml.	Storage time, wk.	$Q_{H^+}^{ch}$				
			Unfrozen control	Frozen			
				Sucrose concentration, *M*			
				0	0.05	0.5	1.0
Wheat	0.62	0	570	230	330	580	580
		3	—	100	160	590	480
	0.36	0	970	0	700	1050	1030
		2	—	0	470	720	780
N.Z. spinach	0.24	0	1100	0	880	1020	1190
		2	—	0	640	950	1140
Millet	0.45	0	1360	930	1160	1210	1220
		3	—	570	970	760	1170
Flax	0.18	0	2010	950	1740	2000	2000
		3	—	660	1360	1460	1610
Swiss chard	0.42	0	1770	1950	1840	1810	1940
		3	—	1100	1440	1620	1720

weeks' storage. Greatest retention of activity during storage was obtained in the presence of 0.5 *M* −1.0 *M* sucrose, and even 0.05 *M* sucrose had a pronounced stabilizing effect. Two of the grana suspensions showed zero activity when frozen and thawed in the absence of sucrose.

Mention should be made of destructive effects of extremely low temperatures which were observed with chloroplast suspensions that had been stored for some time at −40° C. When samples that had been "snap-frozen" at −75° C. and stored for a few weeks at −40° C. were placed in dry ice baths (−75° C.) prior to thawing and testing, a large decrease in photochemical activity resulted:

Time at −75° C., min.	0	20	40
Experiment No. 1, $Q_{H^+}^{ch}$	1290	970	500
Experiment No. 2, $Q_{H^+}^{ch}$	1490	790	880

Chloroplasts that have been stored at −40° C. thus should not be subjected to −75° C. before testing, despite the fact that chloroplasts can be stored for weeks at −75° C. without deleterious effects. The low temperature injury referred to above is associated with the appearance of dark acid production (2) in spinach chloroplast suspensions.

Stabilization of Activity by Sucrose Substitutes

Preliminary experiments indicated that 0.5 *M* sucrose can be replaced as a stabilizer by 0.5 *M* maltose, dextrose, and xylose. Sodium alginate (0.5%), 0.02 *M* asparagine, 5% glycine, and 0.1% ascorbic acid induced greater deterioration than occurred in chloroplasts frozen in water. Half-molar sorbitol, 0.4 *M* mannitol, 0.5 *M* cysteine, 0.02 *M* citrate, malate, or oxalate, 10% propylene glycol, and 15% methanol, did not show damaging effects, but in single tests were less effective than 0.5 *M* sucrose. Among the above compounds, alginic acid is widely used in reducing crystal size in ice cream; 0.1% ascorbic acid has beneficial effects in the preservation of frozen fruits (16); dilute methanol, propylene glycol, and glycerol solutions have been reported previously to reduce deterioration in unfrozen chloroplasts (4, 8, 11).

Photochemical Activity of Thawed Chloroplasts With Different Oxidants

Table IV shows that the highest initial rates of oxygen production by isolated chloroplasts are obtained with Hill solution, followed by ferricyanide, quinone, and chromate; freezing and thawing the chloroplasts in 0.5 *M* sucrose did not cause appreciable deterioration, regardless of the oxidants or methods used in estimating photochemical activity.

Lyophilization

An extensive and detailed study of chloroplast preservation by lyophilization revealed remarkable differences in the retention of photochemical activity between successively lyophilized samples. Certain factors were found to influence the deterioration that accompanied lyophilization: the presence of

TABLE IV

PHOTOCHEMICAL ACTIVITY OF CRUDE SPINACH CHLOROPLASTS IN DIFFERENT OXIDANTS, BEFORE AND AFTER BRIEF STORAGE AT −40° C.

(Activity measurements were made manometrically in saturating light, 10° C., N_2. Chlorophyll, 0.2 mgm. per vessel)

Sample	Photochemical activity, $Q_{O_2}^{Ch}$			
	Hill solution*	Ferricyanide*	Quinone*	Chromate*
Fresh chloroplasts	1690	1080	890	700
Thawed chloroplasts	1510	1080	910	700

** One milligram of quinone; 2.5 ml. of Hill solution, 0.03 M ferricyanide, or 0.03 M chromate were employed as oxidants.*

0.5 *M* sucrose was essential—otherwise the dried chloroplasts were completely inactive when reconstituted; retention of activity was improved by maintaining the ampoules in ice water throughout the freeze-drying operation; the losses of activity associated with dehydration occurred mainly during the initial stages of the drying operation (Fig. 5).

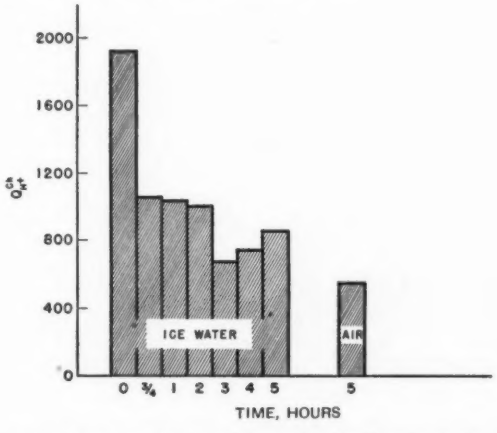


FIG. 5. *Loss of photochemical activity by crude spinach grana during the course of lyophilization in ampoules bathed in ice water or air at 24° C. Estimated dryness after two hours, 95% to 100%. Chlorophyll content: 0.25 to 0.28 mgm. per vessel.*

The wide differences in retention of activity reported in Figs. 6 and 7 cannot be explained, however, by variations of known factors in the freeze-drying operation. The two lots of dried spinach grana, prepared from different sources by the same method, showed 10% and 70% losses of activity respectively. This large discrepancy seems to have been caused by differences in sensitivity to dehydration between the grana preparations. Although the

decreases in activity quotient that occurred during freeze-drying were large and variable, the decreases during subsequent storage of the dry powders at low temperature usually were very small. There were small but significant

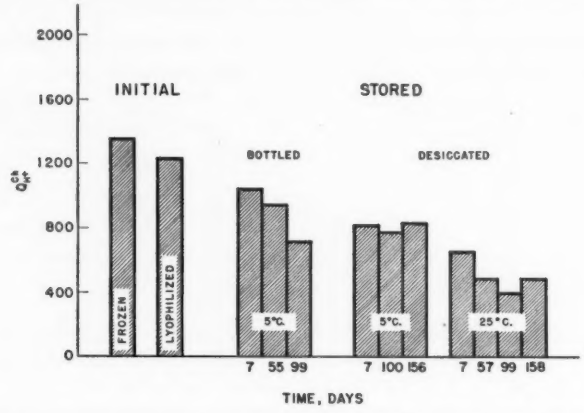


FIG. 6. *Storage deterioration of photochemical activity of crude spinach grana lyophilized by the desiccator method in the presence of 0.5 M sucrose. Chlorophyll content: 0.46 to 0.55 mgm. per vessel.*

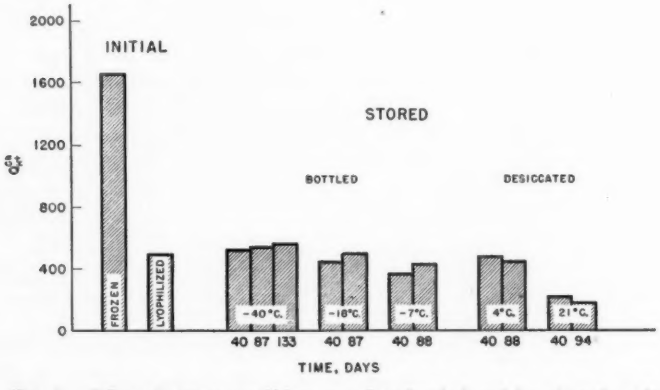


FIG. 7. *Effect of storage conditions on photochemical activity of crude spinach grana lyophilized in 0.5 M sucrose by the desiccator method. Chlorophyll content: 0.40 mgm. per vessel.*

losses (5% – 10%) of chlorophyll during the storage of lyophilized samples, but provided the samples were kept thoroughly dry, the activity quotients remained almost constant at 5° C. and lower storage temperatures. Provision of a nitrogen atmosphere had little effect on the deterioration of photochemical activity that occurred during storage at 20° to 25° C. (Figs. 6, 7). The highest retention of activity in dried grana during one month's storage at −7°, + 4°, and 21° C. was obtained with the driest samples, stored at the

lowest temperature (Table V). Although thorough drying of the chloroplasts and grana usually increases the initial losses of activity, the driest samples are the most stable during subsequent storage.

TABLE V

RETENTION OF PHOTOCHEMICAL ACTIVITY IN VARIOUSLY LYOPHILIZED GRANA DURING ONE MONTH'S STORAGE AT −7° TO 21° C.

(Chlorophyll content, 0.45 to 0.51 mgm. per ml. (or per vessel))

Lyophilization treatment	Initial photochemical activity ($Q_{H^+}^{ch}$ after reconstitution)	Storage temperature, ° C.		
		−7	+4	+21
		% Retention of activity after one month's storage		
Vacuum manifold, 2 hr.	790	70	56	0
Vacuum manifold, 2 hr., + 2 hr. *in vacuo* over P_2O_5	830	76	72	0
Vacuum manifold, 2 hr., + 20 hr. *in vacuo* over P_2O_5	590	93	78	58

Discussion and Summary

The stabilization of photochemical activity in isolated chloroplasts by freezing requires the use of a temperature low enough to prevent deterioration, without damaging the photochemical mechanism. The experiments reported here show that these requirements are met when crude chloroplasts are stored at −40° C. in the presence of sucrose. Deterioration of the photochemical activity after thawing proceeds as rapidly as in freshly isolated chloroplasts, which necessitates the use of successively thawed subsamples in lengthy experiments. However, the variation in activity between separately thawed subsamples ($< 5\%$) is no larger than the variation attributable to the activity measurement itself.

The stabilization of photochemical activity in chloroplasts at low temperatures involves the stabilization of an unknown complement of enzymes. The present observations on chloroplasts include points of similarity with earlier observations on the storage of certain isolated enzymes. In both cases, there is a need for rapid cooling, as well as for very low storage temperatures (9, 15). The activities of isolated chloroplasts and enzymes are both stabilized at low temperatures by sucrose (12, 17). There have also been several reports of significant increases in enzyme activity as a result of freezing (5, 9, 13, 14), similar to the freezing increment observed in the present investigation.

The lyophilization procedures, at least in their present form, are inferior to low temperature storage as a means of preserving chloroplasts for future use. Their principal shortcoming is the large decrease in activity that usually

accompanies drying; when the chloroplasts have been thoroughly dried, there is little additional deterioration during lengthy storage at 0° to 5° C. in an ordinary refrigerator.

References

1. ARNON, D. I. and WHATLEY, F. R. Is chloride a coenzyme of photosynthesis? Science, 110 : 554-556. 1949.
2. CLENDENNING, K. A. and GORHAM, P. R. Photochemical activity of isolated spinach chloroplasts in relation to reaction conditions. Can. J. Research, C, 28 : 78-101. 1950.
3. CLENDENNING, K. A. and GORHAM, P. R. Photochemical activity of chloroplasts in relation to source and previous history. Can. J. Research, C, 28 : 114-139. 1950.
4. FRENCH, C. S., MILNER, H. W., KOENIG, M. L., and MACDOWELL, F. D. H. The photochemical activity of isolated chloroplasts. Carnegie Inst. Wash. Yearbook, 47 : 91-93. 1948.
5. GARD, M. Gels d'automne, diastases oxydantes et dépérissement de plantes en pleine végétation. Compt. rend. 194 : 1184-1187. 1932.
6. HOLT, A. S. and FRENCH, C. S. The photochemical production of oxygen and hydrogen ion by isolated chloroplasts. Arch. Biochem. 19 : 25-43. 1946.
7. HOLT, A. S. and FRENCH, C. S. Oxygen production by illuminated chloroplasts suspended in solutions of oxidants. Arch. Biochem. 19 : 368-378. 1948.
8. HOLT, A. S. and FRENCH, C. S. The photochemical liberation of oxygen from water by isolated chloroplasts. Chap. 14. Photosynthesis in plants, *edited by* J. Franck and W. E. Loomis, Iowa State College Press, Ames, Iowa. 1949.
9. KIERMEIR, F. Der Einfluss des Gefrierens auf die Wirksamkeit von Fermenten. Biochem. Z. 318 : 275-296. 1947.
10. MACDOWELL, F. D. H. The effects of some inhibitors of photosynthesis upon the photochemical reduction of a dye by isolated chloroplasts. Plant Physiol. 24 : 462-480. 1949.
11. MILNER, H. W., LAWRENCE, N. S., KOENIG, M. L., and FRENCH, C. S. The photochemical activity of disintegrated chloroplasts. Carnegie Inst. Wash. Yearbook, 48 : 88-89. 1949.
12. NEUBERG, C. and ROBERTS, I. S. Invertase. Sugar Research Foundation, (N.Y.). Sci. Rept. Ser. No. 4. 1946.
13. NORD, F. F. Physikalisch – chemische Vorgänge bei Enzymreaktionem. Ergeb. Enzymforsch. 1 : 77-112. 1932.
14. NORD, F. F. Beziehung zwischen Tieftemperaturforschung und Kolloidchemie. Naturwissenschaften, 24 : 481-486. 1936.
15. SIZER, I. W. and JOSEPHSON, E. S. Kinetics as a function of temperature of lipase, trypsin, and invertase activity from −70° to 50° C. (−94° to 122° F.). Food Research, 7 : 201-209. 1942.
16. STRACHAN, C. C. and MOYLS, A. W. Ascorbic, citric and dihydroxymaleic acids as antioxidants in frozen food packs. Quick Frozen Foods, 12 (1) : 51. 1949.
17. TRESSLER, D. K. Preventing changes in stored frozen foods. Food Industries, 5 : 410, 432. 1933.

STUDIES OF CANADIAN THELEPHORACEAE

VI. THE *PENIOPHORA RIMICOLA* GROUP[1]

By H. S. Jackson[2]

Abstract

In the following contribution four species of *Peniophora* are described, illustrated, and discussed, which appear to represent a closely related group. These are *Peniophora rimicola* (Karst.) Höhn. & Litsch., *P. pruinosa* (Pat.) comb. nov., *P. pulverulenta* (Litsch.) comb. nov., and *P. praeterita* proposed as a new species. These species have several characters in common. All are mucous-gelatinous when fresh, drying to a vernicose film. In all of them simple, capitate, or apically lobed cystidioles are present and the spores show some degree of roughening when mounted in Melzer's solution. All but one, *P. pulverulenta*, have long emergent cystidia which may be slightly thickened at the base.

Introduction

For some years European students of the Thelephoraceae have recognized two species of *Peniophora* which possess characters indicating close relationship. These are *Peniophora rimicola* (Karst.) Höhn. & Litsch. and *P. chordalis* Höhn. & Litsch. They were included by Bourdot & Galzin (1) in subsection B, Ceraceae of their section Glœocystidiales, but appear to be quite unrelated to other species included in that section. Neither of these species had been recognized in North America at the time of Burt's (2) treatment of the genus. More recently Rogers (3) has correctly recorded *P. rimicola* from Missouri. A considerable number of collections have accumulated in the mycological herbarium at the University of Toronto, some of which are referable to one or the other of the two species listed above, or to two others that seem distinct but which have general characters suggestive of relationship. As a result of a careful study of all these collections, in comparison with type material and other specimens borrowed from various herbaria, it now seems possible and desirable to bring together a preliminary account of the species of this group.

P. rimicola and its immediate relatives, taken as a group, are unrelated to other species included in the genus *Peniophora* as that genus is ordinarily delimited. This group of species, however, because of the manner of growth and the characteristic method of the development of the basidia, often directly from the hyphae on the surface of the fructification, show close relationship to the more characteristic members included in the section Athele of *Corticium* as defined by Bourdot and Galzin. While the authors of this section included

[1] *Manuscript received July 27, 1950.*

Contribution from the Department of Botany, University of Toronto, Toronto, Ont. This study was carried out with the assistance of a grant-in-aid of research furnished by the University of Toronto.

Previous numbers in this series were published in Can. J. Research, C, 26 : 128–139; 143–157. 1948; in C, 27 : 147–156; 241–252. 1949; and in C, 28 : 63–77. 1950.

[2] *The writer is indebted to Miss Elizabeth Ruth Dearden for assistance with the manuscript, to Miss Margaret H. Thomson for the preparation of the latin diagnosis, to Miss Charlotte E. Dill for the preparation of the drawings, and to Dr. D. P. Rogers for critically reading the manuscript.*

some species of doubtful relationship, typical members included there are *Corticium aurora* Berk. and *C. filicinum* Bourd. Species which have so far been recorded from North America, which belong in this relation, are *C. pseudotsugae* Burt (=*C. asserophilum* Litsch.), *C. tulasnelloideum* Höhn. & Litsch., and *C. subinvisabile* Rogers. Some of the species of *Corticium* included by Bourdot and Galzin in their section Ceracea, subsection c, may also properly belong here.

While this is not the place to discuss this group as a whole in any detail, it may be permissible to point out that these species of *Corticium* together with the related species of *Peniophora*, especially those discussed in this paper, possess common characteristics of such a distinctive and fundamental nature as perhaps to justify their ultimate segregation as a distinct genus* or even a group of genera. Judging from the writer's observations, it seems probable that the group will prove to be a large one. Many of the species are very inconspicuous and for this reason have been largely overlooked by earlier students of the group, particularly in North America. It is probable that a majority of the species which will ultimately be brought together in this relation are as yet undescribed.

The species which are to be considered in this contribution would normally be included in the genus *Peniophora* as at present artificially delimited. Three of the four have been previously, but inadequately described, one other is included which appears to be undescribed. Until such time as this group of four species can be considered in relation to the Athele group of Corticiums as a whole it seems best to retain them in *Peniophora*.

The four species have several characteristics in common. All produce fructifications which when fresh are mucous-gelatinous, often with a bluish tint and a pruinose surface suggestive of hoarfrost, finally drying to a hard grayish vernicose film. Sections reveal little character to the subiculum which appears as though made up for the most part of dead gelatinized hyphae, the fructifications perhaps being built up, except for an initial basal layer, by the successive development of more or less horizontal hyphae at the surface of the fructification from which the basidia, cystidia, and cystidioles are derived. In all the species considered, there is a tendency, not always evident in every collection, to the development of cordlike strands which ramify over the surface of the fructification or appear at the margin. The name *P. chordalis*, applied to one of the species, is descriptive of this feature.

Another characteristic common to all four of the species is that the spores, when mounted in water, in Melzer's solution, or in lactic acid, and carefully examined with an oil immersion lens, show some degree of roughening of the walls. This roughening, in all except *P. praeterita*, disappears quickly when potassium hydroxide is used in the mounting fluid and the spore walls appear smooth. Since the use of potassium hydroxide, in connection with the

* *Such a procedure has already been proposed by Dr. D. P. Rogers in an unpublished paper read before the Mycological Society of America at the Philadelphia meeting in 1940.*

examination of dried material of resupinate Basidiomycetes, has come to be a standard practice, this character of roughening of the spore walls has been overlooked by all previous students of the described species.

Still other notable features of this group of species are the variation in the number of sterigmata and spores borne on the basidia, the presence of cystidioles in all of them and typical long emergent cystidia in all but one.

The group as a whole is extremely difficult to study from dried material because of the gelatinous consistency when fresh and the vernicose character when dry. Unless collections are dried quickly and were in active sporulating condition when collected, the basidia are difficult to make out. One is seldom able to view complete basidia or to trace them to the hyphae from which they originate. Occasionally this is possible and then the basidia appear to arise directly from the hyphae (Figs. 3, 4).

The four species discussed may be distinguished by the following key:

Both cystidia and cystidioles present; spore wall markings not arranged in longitudinal spiral lines
  Cystidioles simple or capitate
    Cystidia with thin walls throughout, basidia with 3–5 sterigmata, spores 5.5–9 × 4.8–7 μ, wall markings evident in KOH ..... *P. praeterita*
    Cystidia thickened at base, basidia with 2–4 sterigmata, spores 8–10.5 × 4.5–7 μ, wall markings not evident in KOH ..... *P. rimicola*
  Cystidioles lobed at apex, basidia with 5–6 sterigmata, spores 5–7 × 3–4 μ ..... *P. pruinosa*
Cystidia lacking, cystidioles capitate, spore wall markings in longitudinal spiral lines, spores 8–12 × 4.5–6 μ ..... *P. pulverulenta*

Description and Discussion of Species

Peniophora rimicola (Karst.) Höhn. & Litsch., K. Akad. Wiss. Wien. Math.-Nat. Kl. Sitzungsb. 115, I; 1556. 1906; Bourd. & Galz., Hymén. de Fr. p. 281. 1928; Rogers, Univ. Iowa Studies Nat. Hist. 17 : 31. 1935. (Fig. 1).

Corticium rimicolum Karst., Hedwigia 35 : 45. 1896 (!!); Bidr. Känned Finl. Nat. Folk 62 : 93. 1903; Sacc. & Syd., Syll. Fung. 14 : 221. 1899.

Fructification widely effused, thin, when fresh mucous-gelatinous, hyaline or bluish gray, drying to a whitish or gray semitransparent vernicose film; surface pruinose under a lens due to cystidia and emergent basidia, usually firmly adnate, sometimes with mucous threadlike strands fertile on all sides, extending over the surface of the fructification or at the margin; subiculum somewhat obscure, lower hyphae more or less horizontal, parallel with substratum, upper hyphae interwoven, with clamps, both tending to become gelatinized; cystidia cylindric, 50–125 μ long by 4–6 μ wide in upper half, often broader below to 10–15 μ, walls thin above, usually appreciably thickened below, these thickened portions in old collections remaining as tubules; cystidioles few, cylindrical or slightly clavate, 25–35 × 2–3μ, simple or more commonly capitate at apex to 3–5μ; basidia cylindric or somewhat clavate, 15–25 × 7.5–8 μ, base usually obscure, sometimes bifurcate, bearing two, three, or four short, arcuate sterigmata, 3–4 μ long; basidiospores broadly ellipsoid,

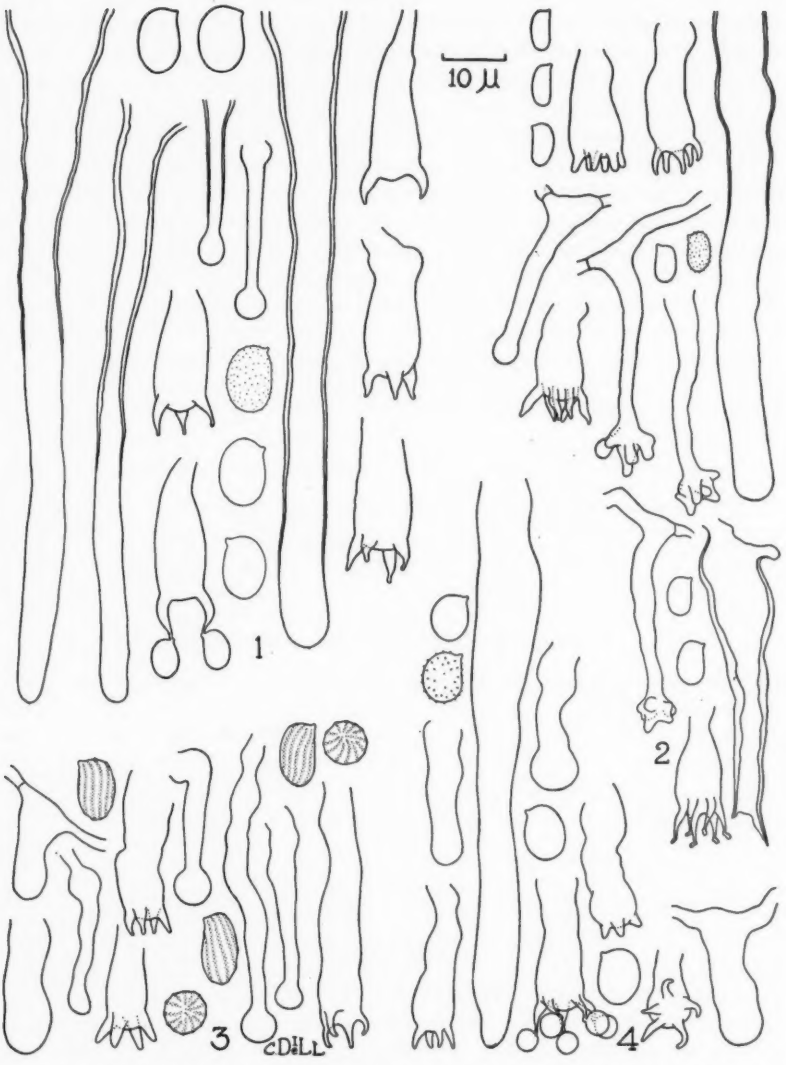


FIG. 1. Peniophora rimicola, *cystidia, cystidioles, and basidia to the left drawn from the type, those to the right from Ontario 14012.* FIG. 2. Peniophora pruinosa, *in part drawn from type of* P. subgelatinosa *and in part from Litsch. 27.* FIG. 3. Peniophora pulverulenta, *drawn from Ontario collections Nos. 17582 and 18720.* FIG. 4. Peniophora praeterita, *drawn from Ontario Nos. 17597 type, and 17599. Reproduced at a magnification of approximately 1000×.*

8–10.5 × 4.5–7 μ, slightly depressed on one side with lateral apiculus, walls smooth in potassium hydroxide but finely asperulate in water and Melzer's solution, nonamyloid.

Specimens examined:

Ontario:* On wood of *Fraxinus* and on *Fomes conchatus*, Bear Island, Lake Timagami, July 30, 1943, *18675*; on bark of *Populus*, R. Biggs. Aug. 5, 1935, *14012*; on deciduous wood, R. F. Cain, Aug. 28, 1935, *8956*; on stems *Rubus* sp., Hogg's Hollow, N. Toronto, Sept. 16, 1944, *19121*; on wood of deciduous tree, July 9, 1946, *20554*; on wood of *Salix*, Sunnybrook Park, Don Valley, Toronto, Sept. 29, 1942, *18213*; on *Alnus*, swamp N. of Mt. Albert, York Co., Sept. 25, 1936, *12499*; Upper Don Valley, Toronto, July 22, 1946, *20667;* on *Acer*, Nashville, York Co., R. F. Cain, Nov. 1, 1948, *23202*.

British Columbia: On wood of *Acer macrophyllum*, Beaver Lake, V.I., July 2, 1948.

Missouri: On decorticated *Ulmus*, Turner's Station near Springfield, A. M. Rogers, Oct. 2, 1933.

Oregon: On *Quercus Garryana*, Corvallis, A. M. & D. P. Rogers *817*, 1936; On *Acer macrophyllum*, D. P. Rogers *731*, Apr. 19, 1937.

Washington: On *Acer*, Lake Crescent, A. H. Smith *14302*, June 13, 1939.

Finland: Auf *Populus*, Rinde und Holz, Mustiala, Oct. 1895, P. Karsten, **type**, FH–H; in cort. *Populi tremulae*, Mustiala, P. Karsten, FH–B.

Brazil: Sao Leopoldo, Sept. 1932, J. Rick (as *Tulasnella metallica* Rick) MO *71742*.

P. rimicola is the first of the group of four species under discussion to have been described and appears to have a world wide distribution. The name evidently refers to the occurrence of the original specimen in cracks in the bark of *Populus*; though the species is more commonly encountered broadly effused on decorticated wood.

This species was first recorded from North America by Rogers (3, p. 31) based on a single collection made in Missouri. He included as synonyms *P. chordalis* Höhn. & Litsch. and *Tulasnella metallica* Rick. As noted below, we consider *P. pruinosa* (= *P. chordalis*) amply distinct. The specimen of *T. metallica* (MO, 71742) studied by Rogers and assumed, at the time, to be authentic proved later to be a misidentification by Rick and quite different from his type which is *Ceratobasidium atratum* (Bres.) Rogers (cf. Rogers & Jackson (4, p. 272)).

* *Unless otherwise noted collections were made by the author. All specimens recorded, other than the types of P. rimicola, P. pruinosa, and P. chordalis, and a few other extralimital collections, are represented in the mycological herbarium of the University of Toronto. The herbarium abbreviations, indicating where specimens are deposited, are as proposed in the report on the standardization of herbarium abbreviations, Chronica Botanica, 5 : 142–150. 1939. Numbers of Ontario collections are those of the University of Toronto Herbarium, TRT.*

In the Von Höhnel herbarium at the Farlow there is another specimen, in addition to those listed, which proves to be *P. rimicola*. This specimen, which is without data, is marked *Coniophora Kalchbrenneri* n.sp. Bres. in Herb. Berol.—ex herb. Winter—sub *Corticium atrocinereum*. On the packet is a note—"Ist ein *Peniophora* mit kugel sporen". We have been unable to determine that the species was ever published. According to Miss E. M. Wakefield (in litt. Dec. 31, 1946, Feb. 18, 1947) *Peniophora atrocinerea* Massee (= *Cort. atrocinereum* Kalch. MS) is a pale form of *Stereum Schomburgkii* Berk. and quite unrelated to *P. rimicola*.

The cystidia in *P. rimicola* are like those of *P. pruinosa*, tending to have thickened walls toward the base and in both species these thickened bases, in old collections, are retained as tubules after the thin upper portion of the cystidium has disappeared.

The number of spores on the basidia varies from two to four and there appears to be a correlation between spore size and the number of sterigmata. In the two Karsten collections the spores measure 8.5–10.5 × 6–7 μ and the sterigmata number from two to four with three as the most common number. A comparable collection is Ontario *8956* in which the basidia have two to three sterigmata most commonly, with spore size as in the type. American collections in general, however, have spores slightly smaller and narrower, 8–10 × 4.5–5.5 μ, and in many of these three to four spored basidia predominate. In contrast, *P. pruinosa* has basidia with five to six sterigmata and the spores are consistently smaller, 5–7 × 3–4 μ.

The cystidioles in *P. rimicola* are simple or more commonly capitate in contrast to the lobed cystidioles characteristic of *P. pruinosa*.

Peniophora pruinosa (Pat.) comb. nov. (Fig. 2)

Corticium pruinosum Pat., Cat. rais. pl. cell. Tunisie p. 60, 1897 (!!); Sacc. & Syd., Syll. Fung. 14 : 222. 1899.

Peniophora chordalis Höhn. & Litsch., K. Akad. Wiss. Wien Math.-Nat. Kl. Sitzungsb. 115, I : 1598. 1906 (!!); Bourd. & Galz. Soc. Mycol. Fr. Bul. 28: 382. 1913. Hymén. de Fr. p. 280. 1928.

Peniophora subgelatinosa Litsch., Österr. Bot. Zeitschr. 77 : 128. 1928 (!!).

The general features of the fructification and of the cystidia are essentially as described for *P. rimicola*; cystidioles cylindric or tapering gradually from base to apex, 25–45–(60) × 2.5–3.5 μ, wall slightly thickened below, apex occasionally simple or capitate when young, more commonly with three to five short blunt lobes; basidia cylindric or cylindric-clavate, 15–25 × 7.5–8 μ, with (four) five to six (seven) short sterigmata; spores ellipsoid, depressed and appearing straight on one side, 5–7 × 3–4 μ, walls thin, appearing smooth in potassium hydroxide but finely asperulate in water or Melzer's solution, non-amyloid.

Specimens examined:

New York: On *Platanus occidentalis*, King's Ravines, N. of Frontenac Point, W. side Cayuga Lake, H. S. Jackson, July 8, 1937.

Austria: Auf morscher Rinde (*Pinus* ?), in der Grossen Klause bei Aspang, Nieder-Österreich, Wechsel, June 2, 1906. FH–H. (**type** of *Peniophora chordalis* Höhn. & Litsch.); on *Quercus*, Georgesberg bei Pr. Kissdorf, Von Höhnel, 1903, FH–H; on *Fagus silvatica*, Lainzer-Tiergarten bei Wein, V. Litschauer, July 12, 1929, FH–B; an morschem Ast von *Quercus* sp., same locality, V. Litschauer *356*, July 7, 1930, ex UPS; on *Fagus*, Kranebittenklamm bei Innsbruck, Tirol, V. Litschauer *27*, Aug. 8, 1929; auf morschen Ast von *Salix caprea*, Ruezbachtal im Stubai, Tirol, V. Litschauer, March 28, 1923, ex herb. Litschauer *36* (**type** of *Peniophora subgelatinosa* Litsch.).

France: sur *Populus*, l'Aveyron, July 1915, Galzin *18291* ex herb. Bourdot *19509*, FH–B; May 1905, Galzin *905* ex herb. Bres. NY.

Tunisia: sur bois de Chêne, El Fedja, Jan. 1893, N. Patouillard (**type** of *Corticium pruinosum* Pat.), FH–P.

Brazil: Sta. Maria, Rio Grande de Sul. Rick. 1936 (as *Kneiffia plumbicolor* Rick n.sp. ined?) FH.

Corticium pruinosum Pat. is quite certainly the same as *P. chordalis*. The cystidia of the two species are alike and the spore size and markings correspond. The type specimen is not in the best of condition but we have seen a few basidia with six sterigmata. Unfortunately we have not been able to demonstrate the presence of the lobed cystidioles but these are often very scarce and perhaps disappear in old specimens. We are quite convinced that the two species are the same and have provided the transfer above.

As noted in the discussion of *P. rimicola* this species differs in the size of spores, in the presence of cystidioles with lobed apex, and in the number of sterigmata and spores borne on the basidia. In. *P. rimicola* the number of sterigmata varies from two to four while in *P. chordalis* there are ordinarily five to six with only occasional ones with four or seven.

As noted above the characteristic lobed cystidioles are extremely scarce in some collections and have not been mentioned by previous authors, except by Litschauer for *P. subgelatinosa*. They have, however, been demonstrated in all the collections assigned to this species, except in the type of *Corticium pruinosum*.

P. chordalis was described and figured as having only four sterigmata. A careful study of the **type** has shown that the basidia for the most part have five sterigmata and one was seen with six. In another collection in the Von Höhnel herbarium the basidia are commonly with five sterigmata with occasional ones with six. While no four-spored basidia were noted in the collections in the Von Höhnel herbarium, a survey of all collections available shows that the sterigmata vary from four to seven. In some, five predominate and in others, six.

The spore wall appears smooth when potassium hydroxide is used in the mounting fluid and shows the least degree of roughening of any of the group when mounted in Melzer's solution. However, careful examination of mounts made in the latter solution shows that the walls are very finely and delicately asperulate.

Bourdot and Galzin give the spore measurement of *P. chordalis* as 6–9 × 3–5 μ which is larger than has been observed in any of the collections recorded above. We have found among specimens determined by Bourdot as that species, one collection referable to *P. pulverulenta*. The spores in the latter species are appreciably longer and this may account for the large measurement recorded by Bourdot and Galzin.

Peniophora subgelatinosa appears to be a form in which the lobed cystidioles are relatively more abundant than usual. Otherwise the characters are the same as here described for *P. pruinosa*. Litschauer gives the spore measurement as 5–8 × 3.5–4 μ. We have not found spores longer than 7 μ in the portion of the **type** of this species available to us and the majority are 6.5 μ or less.

Peniophora pulverulenta (Litsch.) comb. nov. (Fig. 3)

Corticium pulverulentum Litsch. Österr. Bot. Zeits. 88 : 112. 1939.

Fructification widely effused, when fresh mucous-gelatinous, hyaline or bluish gray, drying to a whitish, gray to cinereous semitransparent varnishlike incrustation, surface becoming pruinose and somewhat pulverulent under lens; subiculum thin, 20–30 μ, obscure, hyphae irregularly horizontal walls thin, soon gelatinizing; large cystidia absent; cystidioles few, simple, capitate, 16–25 × 3–3.5 μ, enlarged to 4.5–5 μ at apex, wall thin; basidia cylindric or broadly clavate, 20–25 × 5.5–8 μ bearing usually four, rarely two, three, or five subulate, arcuate sterigmata, 4–5 μ long; basidiospores ellipsoid, 8–12 × 4.5–6 μ, slightly depressed on one side with lateral apiculus, walls thin or slightly thickened, smooth in potassium hydroxide, in water, lactic acid, or Melzer's solution adorned with longitudinal markings 0.5–1 μ apart, arranged in a broad spiral, nonamyloid.

Specimens examined:

Ontario: On bark or wood of deciduous trees, Paradis' Bay, W. shore Lake Timagami, Aug. 7, 1937, *16698*; Woods W. of Maple, York Co., Oct. 28, 1939, *17710*, Oct. 11, 1941, *17582*, Oct. 9, 1943, *18720*, Sept. 21, 1947, *22437*, Nov. 6, 1948, *23200*; woods N. of Richmond Hill, York Co., Oct. 25, 1942, *18656*, Sept. 27, 1947, *22438*; on bark of *Tsuga canadensis*, woods near Sunnybrook Park, Toronto, Sept. 23, 1944, *19122*.

Austria: On morschem Laubholz (*Fraxinus excelsior* L.), Nied.-Österr., beim Lunzersea, Sept. 16, 1930. V. Litschauer, **type**.

France: On *Cornus* (cornouiller) Labastide-Pradines, L'Aveyron, June 5, 1913, M. Galzin *13127*, herb. Bourdot *13954*, Lloyd herb. *33472*, BPI (as *P. chordalis* Hohn. & Litsch.)

The proper determination of this species was long delayed and our Ontario collections have been carried for several years under an herbarium name. My associate, Miss E. R. Dearden, was responsible for calling to my attention the fact that the spores of *Corticium pulverulentum* Litsch. showed the spiral marking which was the chief characteristic of the supposed undescribed species. A careful examination of the **type** shows all the essential characters of the Ontario collections including the capitate cystidioles. The portion of the **type** available to me is very meager and the description and drawings have been made from Ontario collections.

The species is distinctive because of the spirally marked spores, the presence of capitate cystidioles comparable to those of *P. rimicola* and *P. praeterita*, and the absence of the large cystidia characteristic of the other three species. Neither the spiral marking of the spore wall nor the presence of the capitate cystidioles was noted by Litschauer. The former character is not evident if potassium hydroxide is used in making microscopic mounts and the cystidioles are not abundant and are easily overlooked.

Litschauer quite naturally described the species in *Corticium* and it could perhaps well be left there but to do so would separate it generically from what we believe are its immediate relatives. These clearly belong in *Peniophora* as that genus is at present delimited. For this reason the species is here transferred.

The collection from France is one from the herbarium of H. Bourdot in the Lloyd herbarium at Washington, D.C., labeled *Peniophora chordalis* Höhn. & Litsch. In the description of *P. chordalis* furnished by Bourdot and Galzin in their "Hyménomycètes de France", the spore size given (6–9 × 3–5 μ) indicates a longer measurement than I have found in any collection of that species. It seems possible that the spore measurement of the collection cited above may have been included in making up their description.

P. pruinosa = *P. chordalis* has not yet been encountered among collections made in Canada. The only North American collection we have seen is one collected by the writer in New York.

Peniophora praeterita sp. nov. (Fig. 4)

Fructificatio effusa, tenuis, 15–20 μ, viva mucoso-gelatinea, hyalina vel glauco-caerulea, sicca in glauca semi-translucida vernicosa membrana consistens, in superficie sub lente pruinosa; subiculum obscurum, hyphis 2–2.5 μ irregulariter paralleliter currentibus, tunicis tenuibus dein mox gelatineis, nodoso-septatis; cystidia cylindracea, subflexuosa, 60–80 × 6–8.5 μ, tunicis aequaliter tenuibus, projicientibus; cystidiola pauca, cylindracea, simplicia vel capitata, 12–25 × 3–5 μ; basidia cylindracea vel clavata, 18–25 × 6–8.5 μ, 3–5 subulatis, late divergentibus, ad 5 μ longis, sterigmatibus; basidiosporae late ellipsoideae vel subglobosae, 5.5–9 × 4.5–7 μ, tunicis tenuibus hyalinis, minute conspicueque verrucosis, non amyloideis.

Fructification widely effused, thin, 15–20 μ thick, when fresh mucous-gelatinous, hyaline or bluish gray, drying to a whitish gray semi-transparent

vernicose film, surface pruinose under a lens due to cystidia and emergent basidia, firmly adnate; subiculum obscure, thin, hyphae 2–2.5 μ wide, irregularly horizontal, with clamps, walls thin, soon gelatinizing; cystidia cylindrical, somewhat flexuous, 60–80 × 6–8.5 μ, walls uniformly thin, arising directly and at right angles from basal hyphae, projecting for nearly entire length; cystidioles few, cylindrical, simple or capitate, 12–25 × 3–5 μ; basidia cylindrical or clavate, 18–25 × 6–7–8.5 μ bearing three to five, rarely two or six subulate, widely divergent sterigmata to 5 μ long; basidiospores broadly ellipsoid or subglobose, 5.5–9 × 4.5–7 μ, laterally depressed, with prominent apiculus, walls thin or slightly thickened, hyaline, finely and sparsely but prominently verruculose, becoming slowly smooth in potassium hydroxide, nonamyloid.

Specimens examined:

Ontario: On decorticated wood of deciduous trees, including *Acer*, *Betula*, *Ulmus*, *Salix*, and *Populus*; New Durham, Brant Co., R. F. Cain, July 17, 1937, *13714*, Aug. 24, 1937, *13715*, Aug. 30, 1937, *13713*, *13716*; S. of Hatchley, Brant Co., R. F. Cain, Sept. 5, 1941, *18028*, Sept. 22, 1939, *15184*; Benwell Swamp, Gobles, Oxford Co., R. F. Cain, Sept. 24, 1939, *15126*; Silver Lake, Frontenac Co., R. F. Cain, Sept. 1, 1941, *17595*, *17596*, *17597*, **type**, *17598*, *17599*; woods W. of Maple, York Co., Oct. 9, 1943, *18835*; Upper Don Valley, Toronto, July 3, 1946, *20520*, July 22, 1946, *20674*; Camp Billie Bear, Bella Lake, Sept. 12, 1949, *23201*.

Connecticut: On wood of *Ulmus americana*, East Granby, H. G. Eno, Sept. 15, 1939, FP *84871* BPI.

P. praeterita differs from both *P. rimicola* and *P. pruinosa* in the size and shape of the spores and in the large cystidia which show no appreciable thickening at the base.

This is the only species of the four in which the spore markings are sufficiently prominent and persistent to be evident in mounting fluids containing potassium hydroxide. Even in this species the markings very slowly disappear in such mounts after standing for some time.

References

1. Bourdot, H. and Galzin, A. Hyménomycètes de France. Paris. 1928.
2. Burt, E. A. The Thelephoraceae of North America XIV. *Peniophora*. Ann. Missouri Botan. Garden, 12 : 213–357. 1926.
3. Rogers, D. P. Notes on the lower Basidiomycetes. Univ. Iowa Studies Nat. Hist. 17 : 1–43. 1935.
4. Rogers, D. P. and Jackson, H. S. Notes on the synonomy of some North American Thelephoraceae and other resupinates. Farlowia, 1 : 263–336. 1943.

STUDIES IN RACIAL TRENDS AND CONSTANCY IN *CLADOSPORIUM FULVUM* COOKE[1]

By D. L. Bailey[2]

Abstract

Intensive surveys of the physiological races of *Cladosporium fulvum* Cke. have been carried out during the past decade in southwestern Ontario, as a result of which seven such races have been identified. These are separated by differential reactions of the following hosts: *Lycopersicon esculentum* Mill. varieties Potentate, Stirling Castle, Vetomold, V-121, and V-473; *L. pimpinellifolium* (Jusl.) Mill., Vineland, No. 160 and No. 11-22-15 strains; *L. hirsutum* Humb. and Bonpl. and *L. hirsutum* var. *glabratum* Muller. Evidence is presented that only two of these races existed when the surveys began and that the other five have arisen, probably through mutation, during the period under study. There is further a strong suggestion that the stimulus responsible for these mutations is somehow related to the colonization of an incompatible host by a race which remains stable pathogenically while in association with a susceptible host. Comparable mutants have not been encountered under experimentally controlled conditions. A perfect stage has not been found or induced to form in *C. fulvum*. A limited number of types of cultural mutants are produced consistently and these have been studied with respect to variability and the factors initiating them. Such mutants frequently do not form spores and in most instances have a reduced sporing potential; they are unchanged in pathogenicity or are slightly less aggressive than the parent to all the resistant hosts. In mixed cultures of races 1 and 5 and 1 and 7, the components survived several transfer generations both in culture and on a susceptible host.

Introduction

In 1937 Langford (7) reported on studies on physiological specialization in *Cladosporium fulvum* as he found it in those districts of southern Ontario where the production of greenhouse tomatoes is centered. Only two parasitically specialized races were encountered which were differentiated on the basis of the reaction of Stirling Castle or of some four other varieties which reacted in the same manner. To race 1 these varieties were resistant, whereas to race 2 they were susceptible. A strain of *Lycopersicon pimpinellifolium*, the so-called Red Currant tomato, obtained from Vaughan's Seed Store, Chicago, Ill., in 1922, proved generally immune. As an outcome of this work the development of Cladosporium-resistant esculentum tomatoes was undertaken as a co-operative undertaking between the University of Toronto and the Vineland Horticultural Experiment Station and later continued as an Experiment Station project. From this effort to date three new tomato varieties, Vetomold, V-121, and V-473, suitable for greenhouse production, have been given to Ontario growers (3). The present communication reports on the effects which the introduction and general adoption of these varieties have had on the physiological race population of *C. fulvum* in the area concerned and considers the mechanisms by which the changes may have been induced.

[1] *Manuscript received July 27, 1950.*

Contribution from the Department of Botany, University of Toronto. This study was carried out with the assistance of a grant-in-aid of research furnished by the University of Toronto.

[2] *Professor of Plant Pathology, Department of Botany, University of Toronto, and Pathologist, Vineland Horticultural Experiment Station, Vineland Station, Ont.*

Experimental Materials

Physiological races of *C. fulvum* have been recognized on the basis of differential reactions among the following hosts: Red Currant (Vineland strain), Vetomold, V-121, V-473, and Stirling Castle, expressed under conditions which support a normal susceptible reaction on the susceptible variety Potentate. In the later studies, wherever it was possible to do so, two additional Red Currants, obtained through the courtesy of Prof. L. J. Alexander (1) under the numbers 160 and 11-25-15M, as well as *Lycopersicon hirsutum* and *L. hirsutum* var. *glabratum*, have been included for test, although they have proved consistently highly resistant or immune from all the collections tested. Vetomold contains the single dominant gene for immunity from Red Currant which, as was shown by Langford (7), is carried on the fourth chromosome. Likewise V-121 contains the single dominant resistance factor from Red Currant which is carried on the fifth chromosome, while Stirling Castle carries yet another single dominant factor for resistance on the third chromosome. V-473 combines the Stirling Castle factor for resistance with the immune factor from Vetomold. The genetics of resistance in the other Red Currant strains as well as in *L. hirsutum* and the variety *glabratum* is not known at present.

Experimental Methods

With the exception of *L. hirsutum*, which does not set seed with us under either greenhouse or field conditions, and which accordingly has been propagated by cuttings, all the host plants have been grown from seed. Seedlings were grown in 4-in. pots and an attempt was made to have the seeding heavy enough to permit thinning to eight uniform and well spaced seedlings per pot at the time of inoculation. Inoculations were made between the third and fifth leaf stage, the younger stages being preferred under conditions of rapid growth to avoid undue crowding by the time the infections developed. It is essential that vigorous growth be maintained throughout the experimental period, since, if it is slow or becomes seriously checked, the plants harden off and their reaction to the fungus may become quite atypical. As a further precaution against this happening, the terminal growing point is pinched off the plants when they are removed from the inoculation chambers and, whenever such seemed desirable, the plants were watered at that time or a few days later with a nutrient solution or with a 0.8% solution of ammonium nitrate. Humidity was maintained throughout at as high a level as possible and great effort was made to see that it did not fall below about 80% R.H. for any extended periods. Lower humidities may suppress sporulation in compatible host–fungus combinations and are conducive to hardening of the plants as well.

Plants are inoculated by atomizing a spore suspension on the upper and lower leaf surfaces, or, if the amount of inoculum is limited, on the lower surface only. The liquid is allowed to evaporate, after which the plants are placed in cotton-covered inoculation chambers and atomized heavily with

water. The inoculation chambers, which are well hosed before using, are covered with newspaper which is finally thoroughly wet. Satisfactory infections are usually obtained with incubation periods ranging from 24-48 hr., the longer period ordinarily being without injurious effects on the plants provided the temperature has not gone above 90° F. and the plants are returned to the normal greenhouse environment with discretion. This can be done best by uncovering in the evening but, if this is not feasible, the plants may be left partly covered overnight, the inoculation chambers being opened on the shaded side in late afternoon.

The spore suspension used in an inoculation has frequently been prepared from naturally infected leaves collected in an individual greenhouse or from a certain tomato variety within the house. If only a single collection is made from a greenhouse, it is made, if possible, on a completely susceptible variety, two or three dozen leaflets being collected at random throughout the house. This collection is again widely sampled in making a spore suspension of sufficient volume for the projected inoculation. The density of the suspension was not accurately controlled, that represented by slight cloudiness having been found to give satisfactory results. Checks on spore germination were not ordinarily run, but where these seemed desirable, were carried out either in thin films in Syracuse dishes or in shallow drops in Van Tieghem cells at room temperatures. The residue of a greenhouse collection was dried quickly at room temperature and kept in case it became desirable to repeat the inoculation. This was found to be a dependable source of inoculum for a period of six to eight weeks under ordinary room storage.

When a differential host was found exhibiting both a susceptible and a resistant reaction in a greenhouse, the fungus component responsible for the susceptible reaction was isolated by cutting out a sufficient number of susceptible lesions to make a spore suspension adequate to inoculate a complete set of differential hosts in the manner already described. To study the component responsible for a resistant reaction in greenhouse material, tissue isolations were made in a routine manner from the resistant lesions, and usually no difficulty was experienced in isolating the fungus from surface-sterilized excised lesions on potato dextrose or malt agar, provided the lesions were young. This presents no problem in experimental inoculations but isolations from resistant lesions from commercial greenhouses give more capricious results, which are increasingly negative as the crop gets older, the fungus apparently dying out in time within the chlorotic flecks.

Building up sufficient inoculum from the tissue isolation is a simple matter since the fungus grows and sporulates readily on a wide range of ordinary media. Ordinarily, from the initial isolates a spore suspension is made in sterile water and this is used to inoculate heavily slants of dilute (0.25%) malt agar. While a minimum of vegetative development occurs, spores are produced abundantly and are readily washed off this substrate and on it the fungus is stable for much longer periods than on potato dextrose or ordinary 2% malt extract agar.

Stock cultures, as will be discussed more fully under the section dealing with cultural variability in *C. fulvum*, are maintained on sterile leaf mold. When these are increased, two plates are inoculated and from the resulting colonies the spore suspension is made for inoculating whatever number of slants is required. If large amounts of inoculum are required, slants may be made in 6- or 8-oz. bottles or sterilized green-bean pods are easily handled and may be used to advantage.

Inoculations are read when the lesions on the Potentate plants just begin to sporulate freely, which may be from 10 to 20 days after inoculation depending primarily on the temperature, relative humidity and light experience, none of which has been consistently under control.

Physiological Races of *C. fulvum*

In Table I there are summarized the reactions by which are differentiated the seven parasitically specialized races which have been recognized. Races 1 and 2 were differentiated by Langford (7) who also called nonfruiting,

TABLE I

SUMMARY OF THE REACTIONS OF DIFFERENTIAL HOSTS TO PHYSIOLOGICAL RACES OF *C. fulvum*

Host	Reaction of races						
	1*	2*	5	6	7	8	9
Potentate	S**	S	S	S	S	S	S
Vetomold	I**	I	S	S	S	S	I
V-121	R**	R	R	S	S	R	S
Stirling Castle	R	S	R	S	R	S	R
V-473	I	I	R	S	R	S	I
Red Currant, Vineland strain	I	I	R	S	S	R	I
No. 160	I		I	R	R	R	R
H 11-22-15M	I		I	I	I	I	I
L. hirsutum	I		I	I	I	I	I
L. hirsutum var. *glabratum*	I		I	R	R	I	I

** Races 1 and 2 were reported first by Langford (7) who also designated as races 3 and 4 nonsporing, parasitically similar cultural derivatives of races 1 and 2 respectively.*

*** S = susceptible; I = immune; R = resistant.*

parasitically similar, cultural derivatives of them races 3 and 4 respectively. These are excluded from consideration here, as are also the other cultural variants which have been encountered subsequently and whose parasitic capabilities are considered separately. In this summary, for the sake of

simplicity, only the three symbols, S (susceptible), I (immune), and R (resistant) are used to characterize the differential reactions. This does not imply that all the reactions assigned to each of these three classes are identical. Thus, under conditions optimum for fungus development, the susceptible reaction to race 7 is different on Potentate from that of Red Currant. In the case of Potentate the discrete lesions spread rapidly until they coalesce, fruiting is abundant on the lower surface, the fungous layer becomes matted, is grayish colored in the younger stages and on the margin, and buff to tan in the older areas. If high humidity pertains, a lesser but significant amount of fruiting occurs also on the upper surface of infected leaves. Even when infections are numerous, a copious development of fungus takes place well in advance of any necrosis of the leaf, although at times a chlorosis of varying intensity may be seen on the upper surface. In contrast with this, susceptible lesions on Red Currant are much less vigorous in their development. There is little tendency to coalesce, the lesions spread more slowly, fruiting is more restricted, is confined to the lower leaf surfaces, and persists for a shorter time, while chlorosis is more consistently expressed and typically advances beyond the zone of fruiting. The uninhibited susceptible reaction has varied only between these extremes and has been consistently characterized by expanding lesions with relatively abundant fruiting and well maintained host–parasite equilibrium, typically with only a very mild initial chlorosis which passes gradually to necrosis in the older central part of the lesion.

There is considerable variation as well among the resistant reactions, both with respect to the variety and the host–environment complex in specific reactions. The resistant reactions of all the differential hosts have as a common denominator the development of localized hypersensitive lesions within approximately the same time as is required for fruiting in a susceptible reaction, and a sharp reduction or complete suppression of fruiting in the hypersensitive area. The variations are concerned essentially with the size of these flecks and the intensity of the hypersensitive reaction. Thus, under conditions essentially optimum for host and fungus development, the resistant reaction on Stirling Castle consists of relatively small sharply-delimited lesions, yellow to orange in color, the color being more striking on the upper than on the lower leaf surface, and fruiting either lacking or confined to small, compact nonspreading aggregates 1 to 2 mm. in diameter. Under the same conditions the resistant reaction on V-121 is likely to be characterized by larger spreading lesions, less sharply chlorotic and with fruiting more general, though still very much reduced as compared with the susceptible reaction. The resistant reaction on Vineland Red Currant is essentially like that of V-121, whereas that of Red Currant No. 160 is confined to sharply chlorotic flecks which rarely become larger than 1-2 mm. and which only very rarely support any fruiting.

These reactions are sufficiently stable to permit the ready identification of the physiological races under environmental conditions encountered or readily supplied in Southern Ontario (latitude N. 43° 13′, longitude W. 79° 23′).

A certain amount of care has to be exercised, especially in growing the plants and in maintaining a relative humidity which does not fall below 70% for any extended periods of time during the incubation period. The seedlings should be kept growing without check and never allowed to "harden off", since otherwise the reactions become extremely difficult to interpret. It has become our custom, in the determination of physiologic races, not to attempt to read any inoculations in which the Potentate reaction does not fall within the typical susceptible range.

The reactions designated as immune in Table I comprise those in which, as a rule, the inoculated host shows no visible response whatever. At times, however, a few sharply chlorotic flecks may develop which ordinarily do not increase in size or support fungous fruiting. No great conviction is held that this represents a basically different type of reaction from the most resistant ones assigned to the resistant class. Further studies under more rigidly controlled conditions will be necessary to determine this and, in the meantime, the class is maintained as a matter of convenience.

It has not been possible, owing to the lack of controlled conditions, to study the factors which are basic to these fluctuations in host–parasite relations. Langford showed that under sufficiently reduced light experience the resistance of Stirling Castle to race 1 breaks down completely and this has been found to happen also with races 5, 7, 8, and 9. On the other hand, this does not seem to occur with resistances other than the Stirling Castle type. Likewise Schaffnit and Volk (8), Small (9), and others have shown that the disease expression of susceptible varieties is sensitive to a number of factors including nutrition, soil moisture, and relative humidity of the air. Nevertheless, we have found the reaction of well grown seedlings of all the differential hosts sufficiently stable to permit the ready identification of physiological races of *C. fulvum*, throughout a temperature range of approximately 60°-90° F. and any range of light experience which was not obviously limiting to the host.

The Reaction of Differential Hosts to C. fulvum Race 7

To elaborate further the differential reactions indicated in Table I, as well as to give a clearer idea of the range of expression encountered with specific hosts, the reactions of all the differential hosts to race 7, which is now the predominant race occurring in the area under study, will be described.

Potentate gives a typical completely susceptible reaction. Infection is apparent first in Potentate, though only slightly in advance of the other susceptible varieties, with abundant sporulation on the lower side of the lesion and, if the humidity is very high, an appreciable though much reduced fruiting on the upper surface as well. Lesions continue to enlarge and coalesce, the fungus in the advancing margins remaining gray but becoming buff to tan with age. Strikingly little host reaction is apparent until the central area of older lesions finally becomes necrotic (Fig. 1).

If after inoculation the relative humidity drops slightly below that requisite to sporulation, the nonfruiting lesions may continue to expand much

like the fruiting ones, but the colonized tissue soon becomes sharply chlorotic and later necrotic and dry. If the humidity is returned to near saturation, fruiting will usually begin again but is confined to the marginal areas of such lesions. If the initial drop in humidity is still more drastic, colonization of the host is confined to limited, sharply chlorotic nonspreading lesions with greatly reduced fruiting, not unlike a resistant reaction under high humidity.

In June and early July in commercial and experimental greenhouses, not infrequently Potentate will display a sharp chlorosis surrounding *C. fulvum* lesions. Greenhouse temperatures at this time often fall within the range of 90°-100° F. for part of the day and the light experience is at a maximum. Repeated isolation and re-infections have always recovered race 7 from such lesions, which represent apparently an environmental response.

When Potentate seedlings are grown under subnormal light experience, under local conditions from approximately November to mid-February, and inoculated under these conditions, the initial colonization of the host appears to proceed normally. About the time fruiting would ordinarily begin, however, the whole invaded area collapses leaving a paper-thin lesion which usually neither spreads nor sporulates but from which the fungus can be recovered in culture more readily and within a shorter time than from resistant flecks. This reaction has been termed "susceptible killing". If this represents the true hypersensitive reaction of tomato to *C. fulvum* the resistant reaction would appear to be of quite a different type.

The reactions of Vetomold and V-121 to race 7 are alike and not significantly different from that of Potentate. In commercial houses the three reactions cannot be differentiated, but in artificial inoculations the fungus develops a bit more slowly and infection is frequently a bit lighter on V-121 than on Vetomold or Potentate. The range of reactions encountered is also essentially the same as in Potentate.

Vineland Red Currant is completely susceptible to race 7 and under optimum conditions the lesions for a time continue to spread and sporulate heavily. There is not the same capacity as in Potentate, Vetomold, and V-121 for sustained sporulation, which may, however, be merely a reflection of the much thinner and less robust leaf structure. Frequently Red Currant lesions will have ceased sporulating and become necrotic and dry except for the advancing margin, when lesions of the same age on Potentate will still be fruiting abundantly throughout the area colonized. In Red Currant too there is a marked tendency for the leaves to become chlorotic and drop if infection is too heavy or conditions are otherwise unsuitable. Susceptible killing may be typically expressed (Fig. 5).

Red Currant No. 160 is very highly resistant to race 7, but quite consistently a relatively small number of small, sharply delimited yellow flecks develop as a result of artificial inoculations. For some time after these flecks first appear the fungus can be recovered readily from them by tissue isolations.

Red Currant 11-22-15M is virtually immune from race 7. In a very few instances, however, as a result of inoculations during July or August when the humidity was high and the temperature unusually so, a few hypersensitive flecks have developed and from a limited number of these *C. fulvum*, race 7, was recovered.

The reaction of Stirling Castle to race 7 should be classed as moderately resistant. The number of infections is usually as great as on susceptible varieties, but each is centered in a sharply chlorotic area which sometimes continues to expand to a limited extent. Some fruiting is supported but this is localized and limited usually to the under surface of a part only of the chlorotic area. Under very favorable conditions sporulation is more abundant and in a few instances under fall crop conditions has been observed to occur to a very limited extent on the upper surface (Figs. 2 and 3).

V-473 seems slightly more resistant to race 7 than does Stirling Castle. Parallel inoculations yield consistently fewer infections, which are of the same general type but with slightly less fruiting. The reason for this is not apparent, since V-473 contains in addition to the Stirling Castle resistance factor only the immune factor from Red Currant, which is not effective against race 7 (Fig. 4).

Lycopersicon hirsutum is typically completely immune from race 7. This species responds to the close confinement at saturation in inoculation chambers by a general chlorosis and dropping of at least the older leaves, or by large blotchy necrotic areas beginning at a leaf tip or at a margin. This response is less pronounced if the inoculation temperatures are kept below 70° F. and if, after the leaves are atomized, inoculated plants are incubated in glass-topped chambers, with the humidity slightly below saturation, and atomized two or three times during the 24-hr. confinement. Occasionally a small number of sharply chlorotic or necrotic flecks develop on such inoculated plants, which appear to be caused by *C. fulvum*, although we have failed on repeated attempts to recover the fungus from them.

L. hirsutum var. *glabratum* is immune or highly resistant to race 7. It is less sensitive to inoculation conditions than *L. hirsutum*, but sheds all but the younger leaves very promptly if conditions are not just right. Ordinarily there is no response to infection but occasionally up to one-third of the inoculated plants will show varying numbers of slowly developing sharply chlorotic lesions which rarely sporulate but from which it is easy to isolate *C. fulvum*.

FIG. 1. *C. fulvum, susceptible reaction on Potentate, under leaf surface;* × *1.*

FIGS. 2 and 3. *Stirling Castle susceptible and resistant reactions respectively.*

FIG. 4. *Variety V-473, one susceptible lesion and several resistant ones;* × *2.*

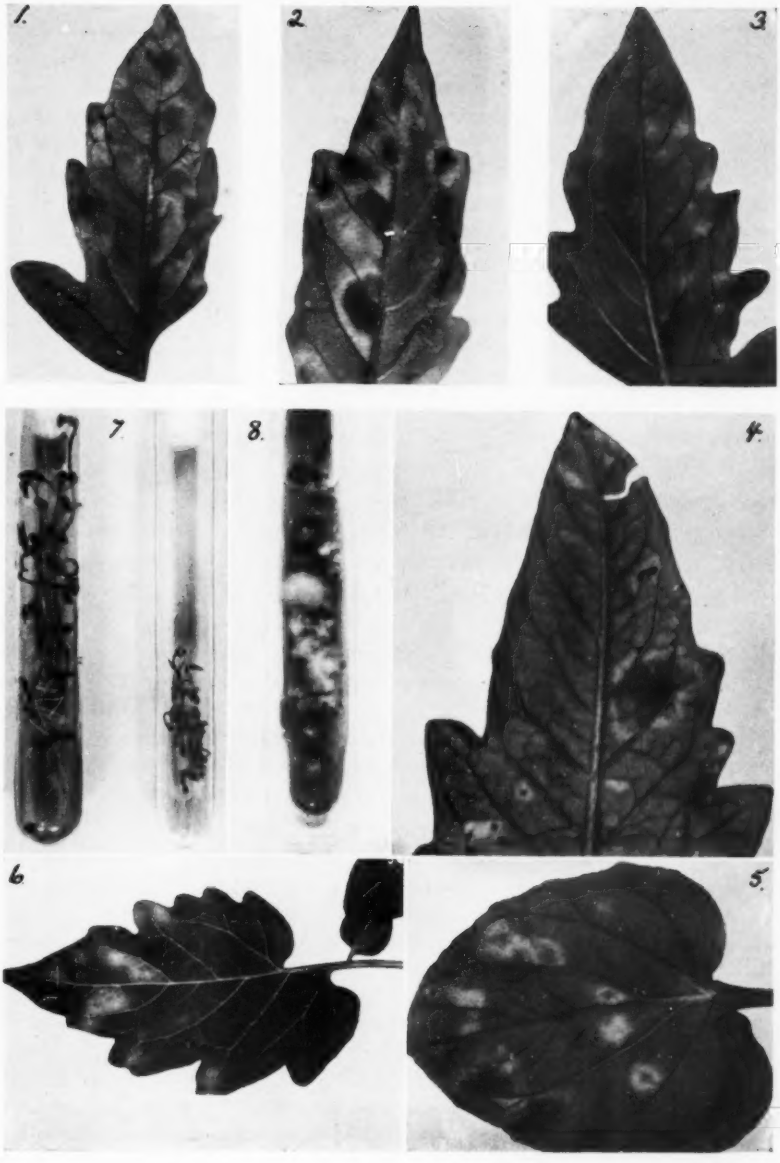
FIG. 5. *Susceptible and resistant reactions on Vineland Red Currant.*

FIG. 6. *The most resistant expression of V-121 to race 7.*

FIG. 7. *Seedlings – Vetomold in the left and Vineland Red Currant in the right tube – on 2% non-nutrient agar, ready for inoculation with C. fulvum.*

FIG. 8. *Four weeks, culture of C. fulvum becoming overgrown with whitish cultural mutants.*

PLATE I



Description of Races Other Than Race 7

With this detailed description of the reactions of the differential hosts to race 7 as a guide, it is believed that little difficulty would be experienced in recognizing the other races. The resistant reaction of V-121, however, is likely to be confusing, because at the two extremes of its expression it may appear like a high degree of resistance or almost complete susceptibility. The expression likely to be encountered under optimum or near-optimum conditions is one of moderate resistance, and is characterized by abundant lesions having sharp chlorosis associated with them from their beginning but still continuing to advance slowly and with slight or, at times, moderate sporulation on the lower surface. The diffuse margin of the chlorotic area and the extent of the fruiting at first appear inconsistent with a resistant reaction, but when contrasted with the complete susceptibility of Potentate the reaction will be recognized as distinct. If relative humidity is maintained at a very high level throughout the incubation period sporulation may be still more vigorous, whereas if it is lowered following infection the reaction becomes highly resistant with sharply chlorotic, nonspreading, nonfruiting lesions (Fig. 6).

It has not been possible to give reactions for race 2 on the newer differentials, inasmuch as the stock cultures of this race were lost before these were worked with and subsequently it has not been encountered.

With the exception of race 9, the races cannot be distinguished on any artificial culture media which have been tried. On both malt extract and potato dextrose agars, race 9 is readily distinguished by the fact that cultures remain grayish-white for a longer time and then develop a chalky green color which is distinctly paler than other normal cultures but equally distinct from the ordinary cultural mutants which arise regularly in all of them. Cultures of race 9 furthermore, do not develop the purple color on dilute malt agar which characterizes the others.

Fluctuations in Physiological Race Population in Local Areas

The general area in which these studies have been carried out is, as Fig. 9 indicates, the southwestern part of Ontario bordering on Lake Erie and the western tip of Lake Ontario. The commercial production of greenhouse tomatoes in Eastern Canada is virtually confined to this area, and within it production tends to be centered discontinuously in several districts, of which the Burlington–Aldershot–Hamilton and Leamington are the most concentrated, with less intensive ones on the Niagara Peninsula, and adjoining Toronto and London. In the Toronto area ordinarily only one crop of tomatoes per year is grown and this is set in the houses sometime in January and harvested mostly from mid-April to mid-June. The fall crop in these houses is likely to be vegetables or chrysanthemums or other floricultural crops. Throughout the rest of the area, the crop sequence varies from year

to year, but frequently two crops of tomatoes are grown, the fall one being set in the house in August and finished off in November or December. In these houses leaf mold is consistently a limiting factor in the production of the

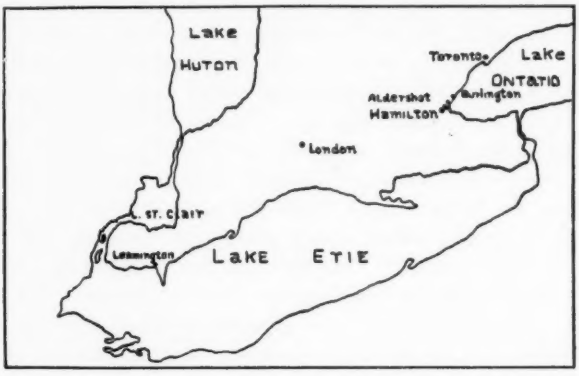


FIG. 9. *Areas of intensive production of greenhouse tomatoes in Southwestern Ontario.*

fall crop and is troublesome but not quite so serious in the spring, when lengthening days and rising temperatures give the grower more latitude with the ventilation.

The first survey related to the physiological races in this area was made by Langford (7) in 1934 and 1935. Only races 1 and 2 were encountered and, although the total number of collections cultured was not large, the indication was that in commercial greenhouses race 1 was predominant.

The first shift in physiological race population was incidental to the introduction of the variety Vetomold (3) in which there had been incorporated the genetic factor from Vineland Red Currant which made it immune from races 1 and 2 of *C. fulvum*. Because of this immunity and the fact that it was well suited to the fall-crop environment, Vetomold was enthusiastically received and rapidly adopted throughout the area wherever a fall crop of tomatoes was grown. Where Vetomold was grown exclusively, leaf mold disappeared completely, whereas where completely susceptible varieties were grown with Vetomold, races 1 and 2 continued to develop, as indicated by limited artificial inoculations and more generally by the complete immunity of Vetomold.

The next shift in physiological race population occurred with the appearance of race 5 to which Vetomold was completely susceptible. This was collected twice on the fall crop of 1939, sporadically on the following spring crop and then quickly built up until it was the only race found on Vetomold and on susceptible varieties growing with it or in houses where it had previously been grown exclusively.

Vineland Red Currant was resistant to race 5, as well as to races 1 and 2, by virtue of the second factor for resistance (7) which it possessed. The variety V-121, which was released in 1941 (3), incorporated this factor and,

because of its resistance to the predominant race 5, was soon grown almost exclusively as the fall crop variety. Growers were urged to maintain it in solid plantings rather than mixed with susceptible varieties and under those conditions V-121 performed very satisfactorily for several crops. Ordinarily, only a light scattering of resistant flecks, which were either nonfruiting or sporulating very lightly, was all that occurred on the plants. The obvious correlation, which was observed repeatedly in large greenhouses, between the number of flecks and the proximity to and the infection carried by susceptible varieties, was convincing evidence of the essentially local character of leaf mold outbreaks. Throughout this period cultures from susceptible varieties yielded only race 5, which was also the only race obtained from the resistant infections on V-121. Once again, however, beginning usually in the third or fourth crop in those houses where the plants were home grown, sporadic infections of the susceptible type began to appear on V-121, which proved to be another race of *C. fulvum*. Race 7, as it was called, was like race 5 except for the susceptibility of Vineland Red Currant and of V-121. In other words, the change in aggressiveness was related to the mechanism of resistance controlled by a single genetic factor located on chromosome 5. This race rapidly dominated the scene until in the physiological race surveys of 1947, 1948, and 1949 it was the only race recovered from commercial houses in which these resistant varieties had been grown.

In 1940 race 6 was found as an isolate from a Stirling Castle-susceptible reaction in the plant breeding greenhouses at the Vineland Horticultural Experiment Station, where all the breeding was done. This race infected Vineland Red Currant, and hence both Vetomold and V-121, and Stirling Castle as well. Race 6 never became general and soon was crowded out even in the Vineland houses by race 7. Unfortunately the stock cultures were also lost and so race 6 has not been available for experimentation. In the fall of 1949, however, what appears from preliminary studies to be race 6 turned up in the same Fonthill greenhouse from which race 5 was first collected. When race 5 was found, the experimentally-minded grower was attempting to overcome what he regarded as a limiting low concentration of carbon dioxide, by closing the houses as tightly as possible and burning charcoal in the aisles. When race 6 was located, culture seemed normal except for an objectionably high level of soluble salts in the soil.

When the resistance factors from Vineland Red Currant were no longer effective, attention was turned to Stirling Castle resistance since it was effective against the widely occurring race 7. Earlier it had been considered desirable to combine the Stirling Castle and Red Currant resistance factors into one variety and hence there was available at this time a combination of the immune factor from Red Currant and the resistance factor of Stirling Castle in a selection which was called V-473. This was released but, because of its small fruit and indifferent yields, it was not grown widely except in the fall crop in houses when leaf mold resistance was of paramount importance.

From the Vineland houses and from only a limited number of other greenhouses, an eighth race of *C. fulvum* has been recovered. Race 8 is like race 5 except that Stirling Castle and V-473 are susceptible. In this case infection capabilities have remained unaltered with respect to Red Currant and V-121 resistance but the mechanism of resistance controlled by the single factor from Stirling Castle located on chromosome 3 (7) is no longer adequate. If race 8 is, as it seems, a derivative of race 5 one would not expect to encounter it generally, inasmuch as race 5 seems to have been eliminated in the majority of greenhouses by the continued cultivation of V-121, which is highly resistant to it. If V-473 becomes generally susceptible the evidence to date suggests that it will probably be through the agency of a race closely related to the now dominant race 7.

For several years the Toronto region was ignored in physiological race surveys because, since only a spring crop was grown, leaf mold was never so serious as it was in the other areas and hence the resistant varieties were not grown as generally or as consistently as elsewhere. Thus a certain number of growers continued to grow Grand Rapids or Sutton's Best-of-all, which were the most popular susceptible varieties before resistant ones were available. From two isolated houses of this sort in 1946 and 1947 race 1 was obtained, at which time race 1 had long since passed out of the picture in the other areas and race 7 was dominant. Race 9 has been collected only once and stems from a collection made near Toronto in 1947 on a variety which could not be identified with certainty but which the grower thought was V-121. After two sets of inoculations had failed to establish the identity of the races present, 45 single spore cultures were initiated from remaining leaves of the original collection. Six of these were normal appearing cultures which on increase and inoculation proved to be typical race 7. The other 39 were alike but easily distinguishable from the other seven by cultural characters. They retained the juvenile grayish white color for a longer period and then developed a chalky green color which was much paler than the others and did not, like these, develop a purple color in dilute malt extract agar. Fourteen of these were tested for pathogenicity and in each case Vetomold proved immune, while V-121 was susceptible, and the reactions of the other were typical of race 1. In other words race 9 is like race 1 except that to race 9 V-121 is susceptible whereas it is resistant to race 1. This seems to fit nicely into the general picture, as a derivation from race 1 in an area where Vetomold was rarely if ever encountered. In this case, however, since V-121 was fairly commonly met with and race 7 was also present in the area at this time, there is the possibility of race 9 having been derived from race 7 through a loss in virulence in relation to Vetomold resistance and to this interpretation the concomitant change in cultural characters may perhaps lend some support.

From this evidence it is apparent that there have been a number of shifts in the physiological race population of *C. fulvum* in the area in question during the 15 years the subject has been under study. The introduction of three resistant varieties, whose resistance in each case depended on a known single

genetic factor, has been followed in each case by the appearance of a single new race of the fungus. In each case the change in pathogenicity which characterized each new race was related only to the new resistant variety to which the local fungus population had but recently been introduced, and no further change was detected until some time after another resistant variety had been introduced. This focuses attention on variability in *C. fulvum* and on the interaction which may occur among its races.

Studies on the Variability of *Cladosporium fulvum*

Variability in Culture

C. fulvum can be isolated without difficulty by tissue isolations from young nonfruiting lesions, or from freshly fruiting ones using either the dry-needle or the spore suspension method. It grows readily on standard malt extract or potato dextrose agars, in each case forming within about two weeks compact circular colonies up to 4 mm. in diameter which are sharply pulvinate, the height being almost as great as the diameter. The mycelium is initially grayish white but, as it becomes more stromatic and spores are formed more abundantly, the surface becomes olive green and later brown with the mycelium beneath it almost black. Colonies may merge or increase radially slowly for some time but, if left undisturbed, will show after a period of about four weeks, when growth has virtually stopped, one or more whitish pin-point areas which enlarge relatively rapidly and finally may cover the entire colony. The atypical mycelium is at first very superficial and may remain relatively superficial and floccose or may become more like the original colony in consistency. If subcultures are made from such a colony with inoculum which samples right through it to the submerged mycelium below, the normal type is usually easily recovered along with the abnormal type, which can typically be subcultured repeatedly without further change. These abnormal types are called mutations in conformity with the very general plant pathological usage of this term to indicate such stable cultural derivatives which arise discontinuously in hyphomycetous species. That they arise in this case through some nuclear change seems indicated by the fact that they were found to appear as readily in cultures derived from one-celled spores, which Langford (7) has shown to be uninucleate, as from two or more-celled spores or from bulk transfers.

The consistency with which these mutants arise and over-run parent cultures makes very difficult the maintainence of stock cultures on either standard potato dextrose or malt extract agars. If the nutrient elements in either of these media are progressively diluted by one-half through a series of five dilutions, the results are interesting especially with reference to the stability of the cultures grown thereon. In Table II the results are summarized from one experiment after culturing at room temperature for 96 days. From these it is apparent that in the fourth and fifth dilutions of both media there is a sharp drop both in the percentage of tubes showing mutants and in the number of mutants per culture; the range in potato dextrose agar being from 100%

TABLE II

THE INFLUENCE OF PROGRESSIVE DILUTION OF MALT AND POTATO DEXTROSE AGAR ON THE STABILITY OF *C. fulvum* CULTURED FOR 96 DAYS AT ROOM TEMPERATURE

Malt agar			Potato dextrose agar		
% Malt	Out of 10 slants		Concentration	Out of 10 slants	
	No. with mutants	Total No. of mutants		No. with mutants	Total No. of mutants
2.5	10	27	Normal	10	31
1.25	10	29	1/2	8	20
0.62	7	8	1/4	8	14
0.31	7	11	1/8	6	11
0.16	4	4	1/16	3	3
0.08	4	4	1/32	2	2

of the cultures with an average of three mutants per culture at full strength, to 20% with an average of a single mutant each at the 1/32 normal dilution. The growth habit changed as well, the stromatic pulvinate colonies of the standard media gradually giving way to progressively flatter, less dense colonies in which, while there was considerably less mycelium developed, spores were formed earlier and in greater profusion, especially in the last three dilutions. This correlation between growth habit, sporing potential, and relative freedom from mutants has been found to hold throughout a wide range of media and at times the concentration of plastic carbohydrate present is apparently of decisive importance. Thus, in the same experiment reported in Table II, cultures were carried also on a soil infusion agar with and without the addition of 0.5% dextrose and, after the 96-day interval, four of the 10 slants with dextrose had mutants, as contrasted with one in those without dextrose.

While no medium has been found which will support a rapid development of the stromatic pulvinate colonies without the early incidence of mutants, a number of substrates, in addition to soil extract agar and dilute malt and potato dextrose agars, have been found on which vegetative development is greatly reduced yet the fungus is viable and satisfactorily stable for extended periods. Among these are sterilized loam, sandy loam, and muck soils, leaf mold, and pieces of tomato leaf petioles, with or without added leaf tissue. Any one of these would presumably be a satisfactory medium for carrying stock cultures, but we have used leaf mold as being somewhat easier to work with than the others. The leaf mold is sieved through not too fine a screen, added to 100 × 15 mm. culture tubes until they are approximately two-thirds filled and to each about 4 cc. of water is added. After the water is absorbed the tubes are plugged and sterilized for two hours at 15-20 lb. pressure. After they have grown on this substrate about two weeks, stock cultures can be stored in the refrigerator for six months without loss in viability and with

very few mutants. The results after 10 months have likewise been highly satisfactory, while in most cases where it has been tried the culture has at least been recovered at the end of a year.

Mutants of C. fulvum

The mutants of *C. fulvum* which appear in culture fall into two fairly distinct groups, the white and the buff types. Among the buff types there is a range from well-colored and heavy sporing ones to light-colored and nonsporing ones, whereas the white mutants seem less variable. Among 25 mutants isolated consecutively from a miscellaneous group of cultures, 19 were buff ones which sporulated more or less abundantly, two were buff ones with little or no sporulation, while four were white mutants sporulating fairly freely.

On malt agar the sporing buff mutants were at first white and fluffy, but soon became a dull greenish buff while the mycelium became dense and felted and produced many spores. Gradually the color of the aerial mycelium became more and more intensely buff, while the reverse side of the colony was a warm dark brown. After four to five weeks quite commonly pale fluffy tufts appeared and at times overgrew the whole colony with a fluffy buff colored mycelium, with very few spores. It is not certain whether these tufts are new mutants or renewed aerial growth under altered conditions. Most subcultures have returned the original mutant only, but on one occasion this pale fluffy mycelium gave a pure white nonsporing mutant which subsequently remained constant.

Six of the 19 sporing buff mutants were monospored and in five of the six exactly the same type of growth was produced. The sixth gave sporing colonies on malt agar which were typical except that the fluffy tufts which developed after about five weeks were even fluffier and more noticeable than usual and were pinkish instead of buff colored.

The two nonsporing buff mutants when grown on malt agar were at first white and fluffy, then pale buff and later a deeper buff color. The aerial mycelium continued much fluffier than that in the sporing forms and did not become dense and felted nor did the greenish color appear. The reverse side of the slant showed a warm brown color similar to that seen in the sporing forms.

The white mutants formed a white fluffy mycelial mat which only after several weeks became dense and felted. The reverse side became dark green. Numerous spores were produced and from each of two cultures 25 single spores were isolated. In the first, all isolations were typical of parent mutant, but, in the second one, two out of the 25 cultures developed into typical sporing buff mutants. In view of the fact that we have no other evidence which suggests a change from the white to buff type, we are inclined to interpret this as the sorting out of a submerged buff type present in the original mass isolate. At the same time it should be observed that usually cultures of *C. fulvum* produce either buff or white mutants and only exceptionally have the two been observed on the same slant.

On potato dextrose agar growth is likewise characteristic for each type of mutant. The buff types do not ordinarily produce their buff color, but become instead gray to lilac tinted, although the buff color is sometimes observed at the top of old slants as the agar dries out. The sporing buff mutants are at first white and fluffy, then pale gray and later deeper gray, while the mycelial mat becomes dense, felted, and rather powdery with the production of numerous spores. After three to four weeks drops of clear liquid exude on the surface of the mat, which are colorless at first but eventually become purple. The same liquid appears to be excreted into the agar which likewise becomes a deep purple around the colony. Tufts of white mycelium appear after about five weeks and overgrow the colony with a white fluffy growth which in turn becomes gray and sporulates.

The nonsporing buff mutants remain white and fluffy on potato dextrose agar for a much longer period than do the sporing types. Eventually pale gray to faintly pink or lilac tones develop and drops of liquid appear which are clear at first but later become pink and then purple. The reverse side of the colony and the agar adjoining it become a deep purple, as in the sporing buff mutants.

The white mutants form a close, felted, white to pale gray mycelial mat on which superficial dark cracks appear. After about four weeks they become whiter and fluffier again, due to renewed growth or to the formation of additional mutants. No drops of liquid are secreted nor does the reverse side become tinged with purple, but is rather pale to dark gray at first and later dark green.

Influence of the pH of the Medium on Mutants

A 2.5% malt extract solution, on which *C. fulvum* grows well, was chosen first as a substrate on which to test the influence of a range of pH reaction on the stability and cultural characters of the mutants. The initial malt extract solution had a pH of 5 and this was adjusted by the addition of appropriate amounts of hydrochloric acid and sodium hydroxide to give an initial pH range of pH 4 to 9. On sterilization these all drifted back to a pH of approximately 6, whereas, when the pH was adjusted aseptically after sterilization, the pH of the alkaline range drifted to pH 7.0 within two weeks. Accordingly a pH range of only pH 4 – 7 was tested, throughout which buff and white mutants and a normal culture displayed the same cultural characteristics as on standard malt agar where the pH is approximately 5. At the end of six weeks the normal culture had produced small compact colonies which had changed from the initial typical olive-green to almost khaki color. The mutant from it showed a few very large fluffy colonies which were deep buff at the edges and a paler buff toward the center. A second unrelated buff mutant produced colonies similar in type but pale buff in color right to the margins. The white mutant had produced a larger number of small colonies which were fluffy and pale gray.

Measurement after six weeks of the final pH of the liquid in each series showed that in every case where the pH was initially 4.0 it had risen to 4.8 or 5.0, while those at an initial pH of 5 had remained at that value or changed only by 0.1. In the remaining flasks, where the initial values were pH 5, 7, 7.8, and 9, the corresponding values of the uninoculated controls after 14 days were pH 5.8, 6.4, 7.0, 7.0, and the final pH's in the inoculated series were around 5.5 for the first two pH's, and around 7.0 in the other two, with the exception of the white mutant where the final values were 5.3 and 5.5.

A second experiment was run with the same group of cultures, namely a normal strain, a nonsporing buff mutant from this strain, an unrelated nonsporing buff mutant and a white sporing mutant unrelated to the other three, using Steinberg's monobasic solution (10) in 1.5% agar as a nutrient substrate. The formula for this solution is sucrose 50 gm., ammonium nitrate 2 gm., potassium dihydrogen phosphate 0.75 gm.; magnesium sulphate 0.6 gm. per liter of water, and the approximate pH is 4.66. The series as initially set up included the following pH range, 3.5, 4.5, 5.0, 5.5, 6.5, 7.5, and 8.5, but the pH values, particularly the higher ones, dropped during sterilization and the final pH's were measured as 3.2, 4.1, 4.6, 5.5, 6.2, 7.1, and 7.4. The agar at 3.2 remained quite liquid and at 4.1 and 4.6 did not gel quite enough to form slopes. Nevertheless, the tubes at these lower pH values were inoculated and the growth on the surface of the medium observed. To obtain higher pH values a further series was set up using Steinberg's dibasic solution with 1.5% agar. The dibasic solution differs from the monobasic one only in the substitution of 0.48 gm. dipotassium phosphate for the dihydrogen salt and the approximate pH is 7.13. The change in pH was much less pronounced in this instance and final values obtained after sterilization were pH 5.5, 7.8, 8.8, and 9.4. At the two highest values a pronounced color change to a light and a dark brown occurred and in neither of them did any growth occur. Five tubes at each pH value were inoculated with the normal strain and the three mutants under test.

The mutants grew well on the Steinberg nutrients, whereas the normal strain grew badly, starting slowly and remaining inadequate and atypical throughout most of the pH range. At pH's 3.2, 4.1, and 4.6 growth was at first greenish white, chalky and not at all vigorous, but by the end of four weeks had improved considerably and become a more normal dark olive green. At pH 5.5 and 6.2 growth was better than at the lower pH's but was also at first greenish with a chalky white surface. After four weeks the growth was more normal with fairly typical olive green mounded colonies not quite so dark as at the lower pH's. At pH 7.1 growth was chalky at first but with brown tinges amongst the pale green. After three weeks a more normal olive green had been produced, but after four weeks, where the colonies were drying out at their tops, pale gray to black colors were showing. The first mutant was noticed in one of these tubes by the fourth week. At pH's 7.1 and 7.8 the growth was essentially like that at 7.1. Thus, although the growth was subnormal and somewhat atypical throughout, the dark olive green color

prevailed at the lower pH's and became paler as the pH increased, with gray to black tones appearing as the agar dried out. Growth of the three mutants was good throughout the pH range 4.1 to 7.8 and for the first two to three weeks the differences between them were insignificant. After four weeks marked changes had appeared and a final detailed comparison was made at the end of five weeks which is summarized in Table III. From this it will

TABLE III

A COMPARISON OF TWO BUFF AND ONE WHITE MUTANT OF *C. fulvum* AFTER FIVE WEEKS ON STEINBERG'S MONO- AND DIBASIC SOLUTION IN AGAR SLANTS

pH	Mutants	Description (Colors after Ridgway)
	Buff I	Submerged
3.2	Buff II	Submerged
	White	Small rounded colonies, dark greenish olive to dusky olive green
	Buff I	Aerial mycelium fluffy; capucine buff to light ochraceous salmon and orange buff: submerged mycelium dusky olive green; patches of orange where aerial mycelium was in contact with sides of the tube
4.1	Buff II	Ibid; light ochraceous buff to warm buff and seashell pink: submerged mycelium dark greenish olive
	White	Small rounded colonies dusky olive green: fluffy aerial mycelium up the walls of the tube white to seashell pink
	Buff I	Like 4.1
4.6	Buff II	Aerial mycelium fluffy; warm buff to ochraceous buff: submerged mycelium dark greenish olive
	White	Like 4.1
	Buff I	Aerial mycelium fluffy; capucine buff to light ochraceous buff, light ochraceous salmon, warm buff and pinkish buff: reverse side iron gray: colonies 11-12 mm. long
5.5	Buff II	Ibid; orange pink center to seashell pink further out and yellow ocher margin: reverse side saccardo's olive
	White	Aerial mycelium velvety to dense and felted, white to pale olive gray: reverse side light buff to seashell pink with patches of orange at the margins and slate color in the center
	Buff I	Aerial mycelium fluffy, pale salmon to pale pinkish cinnamon with tinges of the buff colors of 5.5; reverse side with patches of orange and gray; colonies 13-20 mm. long
6.2	Buff II	Aerial mycelium as at 5.5; reverse side saccardo's olive with tinges of orange at the margins
	White	Similar to 5.5
	Buff I	Aerial mycelium fluffy; flesh pink to shell pink with clear drops of liquid from colorless to pale hydrangea pink, vinaceous and dark maroon purple; reverse side bay at top to orange and amber brown lower down: colonies 15-20 mm. long
7.1	Buff II	Ibid; seashell pink to pale flesh with tinges of orange buff; reverse side saccardo's olive to orange at the margin in the lower half: upper half bay: drops of clear colorless liquid in one tube
	White	Aerial mycelium like 5.5: reverse side olive citrine with tinges of orange at the margins and at the top bay with shades of seashell pink at the edge
	Buff I	Ibid; shell pink, pale cinnamon pink, pallid vinaceous drab and pale grayish vinaceous: drops as in 7.1: reverse purplish to dark vinaceous and perilla purple at top, orange to amber brown lower down: colonies 16-20 mm. long
7.4	Buff II	As in 7.1 with drops of liquid in all tubes
	White	Similar to 7.1
	Buff I	Similar to 7.4
7.8	Buff II	Aerial mycelium fluffy; shell pink, pale cinnamon pink, pallid vinaceous drab and pale grayish vinaceous color: drops of clear liquid present: reverse side purplish vinaceous to dark vinaceous at the top and orange to amber brown lower down
	White	Similar to 7.1

be seen that, although the buff mutants had developed a pale buff color throughout the whole pH range at the end of two weeks, after five weeks the mycelium of the first mutant had lost its buff color at the higher pH's and became pink to vinaceous with drops of wine to purple colored liquid on the surface and with bay and purple colors on the reverse side, whereas at the lower pH's the mycelium had become a much more intense buff. In the second buff mutant the color changes were correlated with pH range in a similar manner, but in this case, with the exception of the cultures at an initial pH 7.8, the fungus quickly shifted the reaction to pH 3.8 or lower, so that the mycelium was predominantly buff colored throughout all but the highest pH where the purplish colors were produced. The white mutant, on the other hand, did not become buff colored at the lower or at any pH, the aerial mycelium becoming gray to faint pink while the colors showing on the reverse side were very pale and subdued as compared with those of the buff mutants. It is apparent, therefore, that the white mutant is quite distinct from the two buff mutants and a suggestion at least that these are physiologically distinct even though they could not be readily separated on the basis of mycelial color.

The reaction of the white mutant to the lower pH's 3.2 and 4.1 was interesting. While the growth was subnormal, the colonies were discrete, rounded, more or less compact, and so close to the normal green color that it was thought at first they had reverted to the normal strain. From one of these tubes single spore isolations to standard malt agar were made and in every case gave rise to the typical white mutant.

These cultures were kept under observation for nine weeks without any further mutants appearing in any of the three mutant cultures throughout the entire pH range, although after four weeks mutants began to appear and became fairly common in the normal cultures. The changes in the pH of the medium induced by the growth of these mutants are summarized in Table IV.

Changes in Reaction of the Medium Induced by Normal and Mutant Cultures of C. fulvum

Cultures of normal strains of *C. fulvum* of different ages on malt agar were selected at random. The agar was melted out and the pH measured by a glass electrode indicator. The pH of the malt agar of two-weeks-old cultures of the normal type was found to be consistently 5.4 while that of two three-months cultures was 5.6 in each case. That is, the pH had risen only from 5.0 to 5.6 in three months.

The pH of potato dextrose agar when made up was found to be approximately 6.0. Random cultures on this substrate were examined similarly and it was found that in a typical three-weeks-old culture the pH was 7.0. In another similar culture in which the slant was covered with purple mounded growth the mycelial mat was dissected away from the agar which was then divided into two halves, on the upper of which the mold had actually been

TABLE IV

CHANGES IN pH OF THE MEDIUM INDUCED BY THE GROWTH OF THE MUTANTS SUMMARIZED IN TABLE III

Initial pH	Final pH		
	Mutant Buff I	Mutant Buff II	White mutant
7.8	7.0	7.6	8.0
7.8	7.6	7.0	6.5
7.4	7.2	3.6	7.0
7.4	7.2	3.8	6.4
7.4	7.0	3.6	—
7.1	7.2	3.6	6.0
7.1	7.2	3.6	5.4
7.1	5.6	3.8	5.4
6.2	4.8	3.4	5.0
6.2	4.8	3.6	5.0
5.5	4.4	3.4	3.8
5.5	3.4	—	3.7
4.6	3.4	3.4	3.4
4.6	3.2	2.8	3.3
4.1	3.4	2.8	5.4
4.1	3.2	2.8	5.6

growing and on the lower of which no growth had taken place. The pH of the two halves was measured and found to be identical and 7.6. In a four-weeks-old culture with dark olive-green growth the pH was 7.2, whereas in two seven weeks' cultures the pH had risen to 8.4.

The pH drift was followed in potato dextrose agar, incident to growing thereon the same normal strain and its derived nonsporing buff mutant, and the white sporing mutant as of previous experiments, as well as a second sporing buff mutant. To each of a number of tubes containing 10 cc. potato dextrose agar, 0.1 cc. of B. D. H. Universal indicator was added aseptically just before the medium solidified, and slopes were made. The tubes were inoculated in quadruplicate with each of the above strains of *C. fulvum*, as well as two slopes without indicator in each series. The pH of the medium after sterilization was 6.0 and the indicator colored it orange-yellow. The changes in pH which resulted after 3, 5, 7, and 10 weeks of growth are summarized in Table V, in which the maximum change induced in each set of four cultures is compared at each interval. The rate and intensity of the pH change was a function of the amount of growth, but the trends were consistent and the end points essentially the same, at pH 9.5.

From all of these studies it is apparent that potato dextrose agar is strongly buffered against any change toward the acid side, while Steinberg's solution agar resists change toward the alkaline side and malt agar holds the pH in a

TABLE V

CHANGES IN pH INDUCED IN POTATO DEXTROSE AGAR OF INITIAL pH 6.0 BY THE CONTINUED GROWTH THEREON OF VARIOUS STRAINS OF *C. fulvum*

Strain	pH after*			
	3 weeks	5 weeks	7 weeks	10 weeks
I. Normal	7	8.5	9.0	9.5
II. Nonsporing buff mutant derived from I	6	6.5	8.0	9.5
III. Unrelated sporing buff mutant	6**	6	6	9.5
IV. Sporing white mutant	6	7	8.5	9.5

** The highest pH among four slants is recorded in each instance.*

*** Growth from the initial inoculation was poor; no significant change was recorded until spores were produced and initiated vigorous secondary colonies.*

narrow range of approximately pH 5 – 7. Normal strains grow well throughout a wide pH range and mutants appear about as frequently and within comparable periods of time regardless of the reaction. The mutants studied seem as adaptable as normal strains to wide fluctuations in pH and appear remarkably stable.

Pathogenicity of Mutants

Infection experiments with mutant strains have proved unexpectedly difficult and unsatisfactory. Many of the mutants are nonsporing and even those that fruit readily are likely to have a much lower sporing potential than normal cultures.

With the nonsporing cultures, the most satisfactory technique to date has been to homogenize them in a Waring Blendor using the small receptacle. Following this treatment many of the hyphal strands initiate growth on agar and too abundant vegetative development occurs on the leaf surface, following atomizing with the suspension and incubation. The aggressiveness seems low, however, and frequently so few infections result that one wonders if the culture is homogeneous in pathogenicity. The infection type is likewise difficult to interpret in the absence of sporulation. It is hoped to study the infection capabilities of mutants more critically in a controlled environment, but at the moment it should be reported that in all the tests to date the indications are that the pathogenicity of mutant forms is either the same as or less than the normal cultures from which they are derived. No suggestion of increased virulence has been encountered. Where reduced pathogenicity is indicated it seems expressed with all the differential hosts rather than with one or two of them.

Origin of Cultural Mutants

While it might seem that, with cultural mutants as predictable in occurrence as are those of *C. fulvum*, it should be possible to find out what brings them

about, little progress has been made in this direction. Since they have not been found in young cultures, but appear so consistently after about four weeks on standard malt or potato dextrose agars, it was felt they might be related to staling processes. A few experiments were run to test this possibility.

In the first instance an attempt was made to grow the fungus on a liquid medium which was constantly renewed according to a modification of the constant drip method of Wean and Young (11). Although as a nutrient a mixture of 1% dextrose and 1% malt extract in distilled water, on which the fungus grows well in agar, was used, growth was highly unsatisfactory in these disturbed solutions and the method was given up.

C. fulvum was grown next on varying quantities of the same liquid medium with the idea that staling products might become more concentrated when only small amounts of medium were present and thus encourage the earlier formation of mutants. Increasing quantities of the dextrose–malt extract solution from 5, 10, 25, 50, 75, 100, and 150 cc. were added to flasks in quadruplicate series which were then inoculated with as nearly as possible equal amounts of *C. fulvum* spores. At the end of four weeks the liquid in the flasks which initially had had only 5 cc. was nearly evaporated, but large numbers of small mold colonies were present and they were without mutants. In the flasks with 10, 25, and 50 cc. of nutrient there were many colonies which increased progressively in size, but all were without any sign of mutants. In the flasks containing 75, 100, and 150 cc. of liquid, the colonies were larger than in the 50 cc. flasks, were all approximately the same size, and scattered small buff mutants had appeared on the edges of some of the colonies. The flasks were kept for another three weeks during which time the colonies changed from olive-green to brown, but increased very little if at all in size and few if any further mutants were formed. These results are difficult to assess since it is hard to compare the amount and the type of growth in the various cultures. It is interesting, however, that no mutants appeared within seven weeks in any of the cultures where the nutrient was sufficiently limited to check the growth while the colonies were still small. Further, few if any additional mutants occurred in the remaining cultures after these had ceased to grow. This suggests that the mutations occur while the culture is growing and only after vigorous growth has been sustained for a considerable period of time.

The next approach was made by growing *C. fulvum* in flasks containing 150 cc. of the same nutrient solution and replacing this by drawing it off and adding fresh solution with a pipette. It was found that by doing this on the 10th and 20th day mutants developed more quickly and in greater profusion than in the control flasks in which the original liquid remained undisturbed. Instead of one mutant appearing on each of a few colonies, 8 – 12 mutants were produced around the edge of each colony, the first of which were apparent before the end of the third week. After the liquid in these cultures had been

renewed the original colonies began to increase in size with a curious feather-like growth and at the end of six weeks they were still olive green in color, whereas the colonies in the control flask had turned to brown by the end of the fourth week and had ceased to grow.

At the end of the fourth week the liquid was removed from each of the control flasks, divided into two portions with approximately 75 cc. in each and put into sterile flasks. In three series 4 cc. of a sterile 1% dextrose solution was added to one flask while nothing was added to the other half of the solution. In the remaining three series an equal quantity of sterile water was added to one-half while the other remained undiluted. All these flasks were again inoculated as equally as possible with *C. fulvum*. After three weeks, in the flasks containing the original liquid, the spores had germinated to form colonies which were only just visible to the naked eye and these had apparently ceased growing. Where dextrose had been added to the original liquid, there were numerous small olive-green colonies just a little bigger in size. In the flasks where the original liquid was diluted with distilled water, there were numerous sparse olive-green colonies which were just a little bigger again than those on the liquid to which dextrose had been added. All the colonies were the typical olive-green color of young cultures and showed no signs of mutants. The normal colonies on the new substrate were by this time 2-3 mm. in diameter and quite dense. At the end of five weeks there were no further changes in these cultures.

The results of these experiments indicate that cultures of *C. fulvum* do stale on a dextrose–malt extract medium, but they offer no evidence that the accumulation of staling products is the stimulus responsible for the appearance of culture mutants. There is an interesting suggestion that ordinarily the production of mutants is checked about the time conditions are conducive to their formation, through the checking of growth at that time by staling which takes place. Further experiments on the relation between the formation of mutants and certain growth potentials are projected.

Origin of Physiological Races of *C. fulvum*

No perfect stage of *C. fulvum* has been found. Isolates have been mixed promiscuously and have been carried for extended periods of time at low and at room temperatures, on routine media as well as on some special ones, like Westergaard and Mitchell's (12), in which are provided a range of growth promoting substances conducive to fruiting in some fungi. Different physical environments such as different soils, leaf mold and peanut hulls have been tested with different nutrients. The results throughout have been consistently negative. The survival of the imperfect stage for appreciable periods in ordinary greenhouse soil makes it unnecessary to postulate a perfect stage to rationalize the epidemiology of the fungus, but at the same time increases the hazard of overlooking a perfect stage if such were produced in the soil. The writer believes the consistency with which the evidence from the epidemiology of the leaf mold disease and the cultural studies of *C. fulvum* suggests

that a perfect stage is no longer produced in this life cycle should carry more weight than the theoretical possibility that such a stage is still produced with sufficient frequency to be a factor in these studies. At the same time, it is admittedly unfortunate that this problem cannot be resolved with absolute certainty, because the segregation and recombination of factors of pathogenicity incident to the production of the perfect stage in a heterothallic species would account acceptably for the appearance of new biotypes and the survival and distribution of these might be explained on the basis of competition among them and the selective action of the genetically different hosts to which they would have been exposed from time to time.

Maintenance of the Fungus on an Uncongenial Host

It was hoped that if race 1 could be kept associated with an uncongenial host substrate some evidence might be obtained as to its constancy under these conditions.

From two lines of evidence it became apparent that, under conditions suitable for infection, race 1 made significant contact with the protoplasts of Vineland Red Currant even though this variety usually gave no macroscopic evidence of this. In the first instance, it is possible to recover *C. fulvum* from surface-sterilized leaves of Red Currant for as long as eight days following the regular inoculation and incubation, although the fungus is not recovered from similarly inoculated and surface-sterilized checks which have not been similarly incubated. Further, if, following inoculation and incubation at saturation for 48 hr., leaves of Red Currant are cleared and stained in lactophenol – cotton blue, penetration will be found to have occurred quite frequently.

Inoculation experiments with Red Currant seedlings revealed further that the cotyledons were regularly killed by *C. fulvum* and that from such cotyledons the fungus could be recovered following adequate surface sterilization. For example, in one experiment, 17 pairs of cotyledons were surface sterilized for 10 min. in a 2% chlorine solution, following which one cotyledon of each pair was plated on 1.5% agar without nutrient, while the other was stained in cotton blue in lactophenol and examined for penetration. Microscopic examination indicated that penetration had taken place in 11 of the 17 cotyledons examined, while the fungus was recovered in culture from the other cotyledon in 10 of the 11 cases in which penetration was demonstrated.

It was found that seedling development took place quite normally if seeds of Vineland Red Currant were surface sterilized for three minutes in 1/1000 mercuric chloride solution and rinsed well in two changes of sterile water and then transferred to non-nutrient, 1.5% agar slopes in 20 × 150 mm. culture tubes. Twenty-five seeds were placed on each slope and the seedlings were allowed to develop in strong diffuse light until all the cotyledons were formed. By this time (Fig. 7) a fairly dense mat of growth was present in each tube and over this a spore suspension of *C. fulvum* was run from a fine delivery pipette. After a period of approximately two weeks, when a substantial number of cotyledons were obviously infected or killed, all such cotyledons

were taken from a tube, surface sterilized in 2% chlorine solution for 10 min., rinsed in sterile water and transferred to non-nutrient agar plates. The fungus sporulated on these cotyledons within 7 to 10 days and usually within two weeks they were used to make a spore suspension to inoculate another generation of Red Currant seedlings. Usually 12 tubes of seedlings were inoculated at one time, since experience indicated that it required the cotyledons from approximately this number to provide spore suspension adequate for the next inoculation. Since the initial sporulation was on the cotyledons rather than on the non-nutrient agar, by this technique the fungus was maintained in constant association with incompatible host tissue. From time to time, as was convenient, some of the spore suspension prepared for the inoculation of a new set of seedlings was increased by the inoculation of dilute malt slants to the point where these provided sufficient inoculum to test the pathogenicity on a set of differential hosts.

Three series were set up from monosporous cultures of race 1 in July 1947 and were run fairly consistently until December 1949, by which time one had gone through 18 successive inoculations and the other 2, 10, and 12 respectively. Occasional difficulties were encountered, especially during the winter months when light experience was low, with poor infections. Once in a while the spore yield was so low that a generation on non-nutrient agar, or, if the situation was very precarious, on weak malt had to be integrated in the series. As a safeguard against losing a strain completely, part of the spore suspension at each inoculation was added to two tubes of sterile leaf mold.

The more virulent races were found to infect the cotyledons as readily but in essentially the same manner as did race 1 under these experimental conditions. It was impossible therefore, to judge from the symptoms whether in each succeeding inoculation generation any change in infection capabilities had occurred. Nor was there any apparent reason why a more virulent race, should such arise, would find the enhanced virulence of value in competing with the dominant race 1. This necessitated frequent tests for pathogenic changes by inoculating differential hosts in the manner indicated. In these tests, however, the immune factor effective against race 1, which is present in the Vineland Red Currant and is incorporated in Vetomold and V-473, should encourage the expression of any racial component capable of infecting those hosts.

The results have been consistently negative so far as any change in pathogenicity is concerned. In each test of pathogenicity, race 1 and only this race has been recovered. Further, the continued association of race 1 with the incompatible substrate seemed rather to reduce the vigor of the culture, as evidenced by the necessity of increasing the strain from time to time on weak malt agar to obtain enough inoculum to continue the infections.

Since Red Currant is known to have two genetic factors for resistance and these are located in different chromosomes, and since the changes in pathogenicity which have been encountered in nature seem in each case to have been associated with one specific genetic factor, it seemed desirable to replace

Red Currant with Vetomold, which has only one of these factors, in a further experiment of this sort. Vetomold seedlings, as well as those of the other esculentum varieties tested, are not so well adapted to these experimental conditions as are those of Red Currant (Fig. 7). Seedlings are much more robust and, even at greatly reduced numbers, soon become badly crowded. However, by using 10 instead of 25 seedlings per slant and exercising much care with respect to the time of inoculation and isolation, the experiment will work and Vetomold has been carried for as many as eight generations of successive inoculations with race 1. Once again, however, no evidence of change in pathogenicity was encountered.

While the significance of positive results would have been immediately apparent, it is difficult to evaluate the negative ones which have been secured. At first glance they seem to underwrite substantially the thesis that race 1 of *C. fulvum* is remarkably stable in pathogenic characters in association with an incompatible substrate under a wide range of light experience, and it may well be that this is their true import. However, if one looks critically at the opportunity for mutant survival under these experimental conditions, it is evident that much is left to be desired in this respect. At best, competition with the parental strain will be inevitable and severe and, if any sort of antagonism should exist between the two, the opportunity is there for its most acute expression. An attempt was made to investigate the interaction of some *C. fulvum* races.

Racial Interaction in C. fulvum

Mixed inocula or races 1 and 5 and 1 and 7 were prepared from an equal number of slants of the component races in each case and in sufficient amounts to seed two plates of dilute malt agar and to inoculate a set of differential hosts, comprising Potentate, Vetomold, Stirling Castle, V-121, V-473, and Vineland Red Currant. In the resulting infections with races 1 and 5, Potentate, which is susceptible to both races, had a significantly larger number of infections than did Vetomold, whose immunity to race 1 would eliminate all infections from this source. Likewise, Stirling Castle and V-473, which were resistant to both races, had larger numbers of resistant flecks than did V-473 and Red Currant which were resistant to race 5 but immune from race 1. Similar differences were apparent in the mixture of races 1 and 7. Here too, resistant and susceptible reactions were present in roughly equal numbers on V-121, which is susceptible to race 7 and resistant to 1. Throughout these infections, however, there was too much variation to permit the method to be used quantitatively in sorting out the relative proportions of components in mixed infections. A further test will be made of it shortly with the environmental factors more adequately controlled.

In the case of the Potentate plants, which were completely susceptible to all three races, a spore suspension was made in each mixed infection from all the infected leaves, which, after being well shaken, was used to inoculate two more pots of Potentate seedlings. This was repeated for five transfers, after

which a set of differential hosts was again inoculated with the progeny of each of the 1-5 and 1-7 mixtures. The results indicated that the component strains were still present in each mixture, but more than that it was impossible to say.

Races 5 and 7 were grown in mixed culture on dilute malt agar for three culture generations and then returned to differential hosts. Both races survived and there was a suggestion, on which, however, not too much dependence can be placed, that race 7 was somewhat more aggressive than 5 in terms of the V-121 and Red Currant reactions.

While these experiments leave many questions with respect to racial interaction in *C. fulvum* unanswered, they do establish the possibility of race 1 co-existing with race 5 or race 7 on the same susceptible host for as many as five successive transfers. They do not accordingly offer any support to the thesis that the original dominance of race 1, or its subsequent replacement by races 5 and 7, was due to competitive reactions among these races on susceptible hosts. The results suggest that the techniques used in the repeated inoculation of cotyledons should have revealed race 5, if it had been present in any substantial amounts in the inoculum. Since, however, what we were trying to locate were individual spores in which a certain mutation might have occurred, the results give us little basis for estimating the chances of spotting such mutants. The odds against it are enormous. In striking contrast is the high chance of survival if a mutation occurs in the host parasite association as a result of which a hitherto resistant or immune host becomes susceptible. Here a susceptible substrate for the mutant is provided at the same time the competition from less virulent races is eliminated or reduced to a minimum.

Discussion

It is believed that the evidence presented supports the thesis that races 5 to 9 of *C. fulvum* are actually new biotypes which have arisen by mutation and there is further a strong suggestion that the stimulus responsible for those mutations is somehow related to the colonization of an incompatible host by a race which remains stable pathogenically while in association with a susceptible host.

Several lines of evidence converge to suggest that races 5 to 9 are new. In the first instance, none of them was encountered in the original surveys of the area made by Langford (7) and the indicator variety Red Currant remained consistently immune in the several houses in which it was introduced experimentally. Strong additional support is to be found in the universally reported initial immunity of Red Currant wherever its reaction to *C. fulvum* was tested (1, 6, 7). It is interesting and, we believe, significant that the only parasitically specialized race encountered in the initial surveys was race 2, which is characterized by its ability to attack Stirling Castle, and a few other varieties, which incorporated the only factor for resistance known to have been present in locally grown esculentum varieties (7) before the Red Currant derivatives were introduced.

After the development and release of the variety Vetomold there appeared only the one new race, race 5, to which Vetomold was susceptible and Red Currant reacted with the production of sharply necrotic resistant flecks, a single one of which was conspicuous in view of its previous immunity. Not until much later, with the evolution of races 6 and 7, did susceptible reactions appear on Red Currant and these were so strikingly different they could not be confused with the resistant ones. Thus the readiness with which each new race has been spotted on an indicator host variety long before it became general in distribution argues strongly against the possibility of them having been present from the start and having been merely overlooked.

The stability of the initial condition when only susceptible varieties were grown is indicated by the instances already discussed in the Toronto area and by the fact that collections from Quebec, where susceptible varieties only were grown, were all race 1 at a time when race 1 was no longer encountered in Ontario. The same is implied in the consistency with which Vetomold proved initially immune when tried in British Columbia, England, Holland, United States, New Zealand, and Australia. While it has not been possible to compare the races of *C. fulvum* initially present throughout these areas with our races 1 and 2, it seems apparent that no races capable of infecting Red Currant were present, before resistant varieties were introduced. Yet in each of these areas, races to which Vetomold was susceptible appeared after Vetomold was grown in them for some time. For these reasons we believe our races 5 to 9 are new biotypes.

That these new races probably arose as a result of mutations is suggested in the first instance by the lack of any evidence indicating the existence of perfect stage in *C. fulvum* and the very considerable evidence adduced that such is not ordinarily produced in the area under study. They further appear like mutants in that the changes are discontinuous and the derived races are as constant as those from which they spring. Thus each time a new race appeared the change in reaction was from immunity or resistance in a specific host to complete susceptibility, with no intermediate reactions between the two ever having been encountered. Stock monosporous cultures of all these races have been maintained for extended periods of time, which in some cases now reaches four years, without any change in their pathogenicity.

Furthermore, each change in pathogenicity which occurred was related to a resistance mechanism whose expression was governed by a single dominant resistance factor. Thus race 5 is like race 1, except that to it Vetomold is susceptible and Red Currant is resistant, instead of the two of them being immune or, in other words, the immune factor of Red Currant is effective against race 1 but not against race 5. Race 7 is like race 5, except that to it V-121 and Red Currant are susceptible whereas they are resistant to race 5; that is, the second resistance factor of Red Currant is no longer effective. Race 8 is like race 5, except that against it the Stirling Castle resistance factor is no longer effective, so that Stirling Castle and V-473 become susceptible,

while V-121 and Red Currant remain resistant. Race 9 is like race 1, except that V-121 is susceptible rather than resistant, and race 6 is like race 7, except that the Stirling Castle resistance is no longer effective.

The fact that these changes are unidirectional and have occurred repeatedly, but apparently only and always in certain host associations is taken to indicate that they have been induced in some manner by the substrates on which they occurred. The readiness with which hosts are penetrated by fungus races to which they are resistant or immune assures the opportunity for interaction between fungus races and incompatible substrates on an extended scale under natural conditions. A mutant appearing under such circumstances would moreover have an unusually good chance of surviving since competition with other races is automatically suppressed by the resistance to them of the substrate host.

The evidence that the originations in each case have been multiple stems from the fact that in Ontario the epidemiology of *C. fulvum* is closely centered around greenhouses and, since the seed is grown locally and out-of-doors, the inoculum is not seed-borne. So, whatever happens in each of the Toronto, Hamilton–Aldershot–Burlington, Niagara, London, or Leamington tomato growing areas can safely be considered to be taking place independently of the rest. Yet the same thing happened in each of them and even in isolated greenhouses within these areas, where the tomato plants were grown by the grower from seed which he produced: parallel crop histories resulted in each case in the appearance of a fungus race not previously encountered, whose differential reaction was related to the same specific host and was identical. In each case where close observations were made the sequence was as follows: in the first crop, in which a change in the reaction of a variety was noted, infections of the susceptible type were rare and had really to be hunted for; in the next crop they were easy to find but the infection was still light and scattered, while in the third or fourth crop the predominant reaction was the susceptible one.

After each resistant variety was introduced, sooner or later a single new fungus race capable of attacking it appeared and no further new races were found until after another new resistant variety had been introduced. This is taken to mean that in each case the host–parasite association provides the stimulus for the mutation and the shift to a compatible host parasite relationship exhausted the possibilities in this respect. What is implied here in the way of basic mechanism is impossible to say since we do not know what is basic to pathogenicity in *C. fulvum*. There is nothing to suggest a general chemical toxemia in the colonization of a compatible host although the considerable size of the hypersensitive flecks, which characterize many incompatible reactions, and the chlorotic areas, that often accompany susceptible ones, indicates that host cells appreciably beyond the zone of actual colonization are under fungus influence. If this is taken as evidence that pathogenicity is a function of nutrient relations, the sensitivity of Stirling Castle resistance to variation in light experience and of the susceptible reaction

generally to growing conditions might be cited in further support. The simple nutrient requirements for vegetative development of the fungus in pure culture, however, make one pause, although these investigations have not been critical with respect to the requirement of either trace elements or growth promoting substances. While the fungus is extremely vulnerable with respect to moisture relations, such evidence as there is suggests that moisture is more likely to be a limiting factor in connection with the initiation of infection and sporulation than in the colonization of a host after it has been penetrated. Speculation is perhaps more fruitful if one considers pathogenicity a function of the manner in which substances likely to be available to the fungus are metabolized by it. Slight differences in this respect might readily characterize the different races and these might be susceptible to influences exerted only in the parasitic association. Regardless of what physiological processes may be basic to compatibility and determine the existence and degree of incompatibility in the various host–parasite association, it does not seem more difficult to envisage a genetic instability related to them than to such as may be responsible for cultural characteristics or sporulation potential. It will probably be generally conceded that the evidence presented with respect to the appearance of predictable mutations in cultural characters in response to experimental manipulations is good, yet the evidence presented with respect to pathogenic changes as a result of certain host–parasite associations is likely to be suspect, since the mutations concerned appear to be unidirectional and, inasmuch as in response to each change a variety not hitherto attacked becomes susceptible, it smacks too much of an unorthodox inheritance of an acquired character. Some of this objection disappears if one thinks of the change involved as one of altered metabolism and the inclusion of another variety within the host range as incidental to this. If the basic change should be one of enzyme mechanism it might be explained in terms of the shunt metabolism concept as elaborated by Foster (5), implicit in which is the idea that, wherever they occur, mutations must be accompanied by alterations of one sort or another in metabolic processes which can legitimately be considered as acquired characters. In any case, it is believed that if specific host–parasite associations be added to the already substantial list of factors which may influence mutation frequency and if this is investigated more widely, significant advances may be made in our understanding of the evolution of virulence in specialized parasites.

Acknowledgments

The assistance of many students with the routine work of carrying out these physiological race surveys for more than 10 years is deeply appreciated. Further, the contributions of Dr. A. N. Langford, Dr. C. D. McKeen, Mrs. V. P. Webb, Miss Z. Wojic, and Miss J. M. Dickson, as research assistants, are gratefully acknowledged. Nor could the work have been done without the continued generous cooperation of Mr. O. J. Robb and Dr. E. A. Kerr, of the Vineland Horticultural Experiment Station.

References

1. ALEXANDER, L. J., LINCOLN, R. E., and WRIGHT, V. A survey of the genus *Lycopersicon* for resistance to the important tomato diseases occurring in Ohio and Indiana. Plant Disease Reptr. Suppl. 136 : 51-85. 1942.
2. BAILEY, D. L. and LANGFORD, A. N. Can. Plant Disease Survey Ann. Rept. 20 (1940) : 57. 1941.
3. BAILEY, D. L. and LANGFORD, A. N. The development through breeding of greenhouse tomato varieties resistant to leaf mould. Ontario Hort. Expt. Sta. Rept. 1945-46 : 56-60. 1947.
4. BAILEY, D. L. and LANGFORD, A. N. Physiological specialization in *Cladosporium fulvum*. Phytopathology, 38 : 2. 1948.
5. FOSTER, J. W. Chemical activities of fungi. Academic Press Inc. New York. 1949.
6. GUBA, E. F. Bay State, a red forcing tomato bred for resistance to leaf mould. Mass Agr. Expt. Sta., Bull. 393. 1942.
7. LANGFORD, A. N. The parasitism of *Cladosporium fulvum* Cooke and the genetics of resistance to it. Can. J. Research, C, 15 : 108-128. 1937.
8. SCHAFFNIT, E. and VOLK, A. Über den Einfluss der Ernahrung auf die Empfänglichkeit der Pflanzen für Parasiten (I. Teil.) Forsch. Gebiet Pflanzenkrankh. u. Immunität Pflanzenreich, 3 : 1-79. 1927.
9. SMALL, T. Tomato "mildew" of leaf-mould. Cheshunt Expt. and Research Sta. Herts, Ann. Rept. 14 : 45-62. 1929.
10. STEINBERG, R. A. The nutritional requirements of the fungus *Aspergillus niger*. Bull. Torrey Botan. Club, 65 : 81-90. 1935.
11. WEAN, R. E. and YOUNG, R. E. Renewed liquid-cultures of fungi. Phytopathology, 29 : 895-898. 1939.
12. WESTERGAARD, M. and MITCHELL, H. K. *Neurospora* V. A synthetic medium favouring sexual reproduction. Am. J. Botany, 34 : 573-577. 1947.

STUDIES OF COPROPHILOUS ASCOMYCETES

I. *GELASINOSPORA*[1]

By Roy F. Cain[2]

Abstract

Two species of *Gelasinospora* are described for the first time. *G. adjuncta* Cain, a heterothallic species with eight-spored asci, was grown in moist chambers on dung from Germany and Quebec. *G. retispora* Cain, distinguished by means of the large angular pits in the ascospore wall was isolated in culture from seeds of Swiss chard from Holland and from apple twigs in Quebec. *G. tetrasperma* Dowding has been found in moist chambers on dung from Ontario, Quebec, Venezuela, and Germany and isolated from seeds from Quebec. Notes are added regarding the disposition of *Anthostomella destruens* Shear and *Sordaria uvicola* Viala and Marsais.

The Genus *Gelasinospora*

Two species of Pyrenomycetes having ascospores characterized by dark-colored spore walls with pitted sculpturing were described by E. S. Dowding (1) in 1933. For these she erected the new genus *Gelasinospora*. This is closely related to *Neurospora* Shear and Dodge (5) which has longitudinal, wavy ridges on the walls of the ascospores. The species of *Neurospora* produce a conidial stage of the *Monilia* type and spermatia in phialides. In *Gelasinospora tetrasperma* Dowd. and *G. cerealis* Dowd., neither conidia nor spermatia have ever been described to date.

In October 1931, perithecia of *Gelasinospora tetrasperma* were grown on rabbit dung from Thunder Bay District in the laboratory in Toronto. This was recognized as being quite distinct from any known species of *Sordaria* in having the ascospore devoid of any gelatinous sheath or appendages, and in the characteristic marking on the spore wall. These features indicated a closer affinity with members of the Hypocreales. It is now the opinion of the author that most species of *Sordaria* as well as *Neurospora*, *Gelasinospora*, and *Melanospora* should be treated as members of this order in spite of the dark-colored perithecial walls. *Gelasinospora tetrasperma* has since been found on dung from Duchesnay and Lake Mistassini, Que., and from Germany. The type material from near Fort Churchill, Man., has been examined as well as cultures from England referred by Page (2) to *Sordaria fimicola* (four-spored form). It has also been found on seeds from Quebec.

The second species *Gelasinospora cerealis* is known only from the crown of wheat and oats in Manitoba.

In October 1935 a third species of this genus was found on the dung of dog and cow from Germany which was being kept in moist chambers in Toronto.

[1] *Manuscript received July 31, 1950.*

Contribution from the Department of Botany, University of Toronto, Toronto, Ont. This study was carried out with the assistance of a grant in aid of research furnished by the University of Toronto.

[2] *Assistant Professor and Curator, Department of Botany, University of Toronto.*

This was isolated in pure culture. In 1938 the same species appeared in moist chambers on dung from Quebec. This species resembles *G. tetrasperma* but has smaller ascospores and eight-spored asci.

A few years later a fourth species was cultured from Swiss chard seed imported from Holland, by Dr. J. W. Groves at Ottawa. This species differs from the others in having much larger pits in the wall of the ascospore. More recently a second collection of the same species was cultured from apple twigs at Ste. Anne de la Pocatiere, Que., by A. Payette, and forwarded to the writer by Dr. Groves.

Key to the Species of *Gelasinospora*

Asci four-spored ..... *G. tetrasperma*

Asci eight-spored

Pits on ascospore wall small

Ascospores 26-32 × 23-25μ ..... *G. cerealis*

Ascospores smaller 22-27 × 12-15μ ..... *G. adjuncta*

Pits on ascospore wall large ..... *G. retispora*

Gelasinospora tetrasperma Dowd. Can. J. Research, 9 : 294. 1933.

On malt agar producing a rapid and dense growth of mycelium which is white at first, becoming gray or slightly pinkish. Perithecia superficial or with base slightly immersed, pyriform, 430-600 × 270-300μ, black, bare excepting a fairly dense layer of hyphae at base; neck papilliform, black, bare, 150-200μ long and 100-120μ wide, lined with periphyses; wall moderately thick, slightly coriaceous, composed of dark brown angular cells measuring 8-16μ. Asci four-spored, cylindrical, 150-180 × 16-18μ, truncate and distinctly perforate at the apex, tapering below into a fairly short stipe. A few hyaline, swollen cells surrounding the asci but no paraphyses. Ascospores uniseriate, parallel with ascus, ellipsoid, slightly flattened on one side, 23-27 × 13-16μ, broadly rounded at the ends, dark brown, becoming black and opaque, wall uniformly covered with round or slightly elongated pits measuring up to 1μ in diameter.

Developed in moist chambers on dung of ptarmigan, rabbit, horse, and cow from Quebec, Ontario, Manitoba, England, Germany, and Venezuela. Isolated in culture from seeds of *Beta vulgaris* L. (mangels, beets), and *Festuca rubra* L. (red fescue) from Quebec, by Dr. J. W. Groves, Ottawa.

This species is evidently more common in Northern Canada. It has not been found in Southern Ontario or even at Lake Timagami where extensive collections of dung have been studied.

The pits in the wall of the ascospores cannot be seen in the opaque spores. They are plainly visible, however, in the brown immature spores or when the opaque spores are crushed, especially in lactophenol.

Gelasinospora adjuncta sp. nov. (Figs. 15-24)

Peritheciis superficialibus, sparsis vel gregariis, piriformibus, 700-1000 × 450-600μ, nigris, superne denudatis, pilis longis, flexuosis, pallide-brunneis, in parte inferiore vestitis; collo papilliformi vel conico, levi, nigro, denudato, 200-400 × 200μ, periphysibus praedito; membrana perithecii mediocriter crassa, interdum coriacea, fere opaca, e cellulis atro-brunneis, angulatis 10-20μ, constituta. Ascis octosporis, cylindraceis, 200-260 × 18-21μ (interdum 400μ longis), superne truncatis, apice distincte perforatis, basi in stipitem brevem attenuatis, qui post maturitatem elongandam. Sine paraphysibus sed cellulis magnis, hyalinis, vesiculiformibus praeditis. Ascosporis oblique monostichis, ellipsoideis 22-27 × 12-15μ, nigris, opacisque, foraminibus obtectis. Spermatiis ovatis, 2.5 × 2.0μ. Speciebus heterothallicis.

Type: In fimo canino, ex Tamsel, Germania, in laboratorio culta. TRT *23203*.

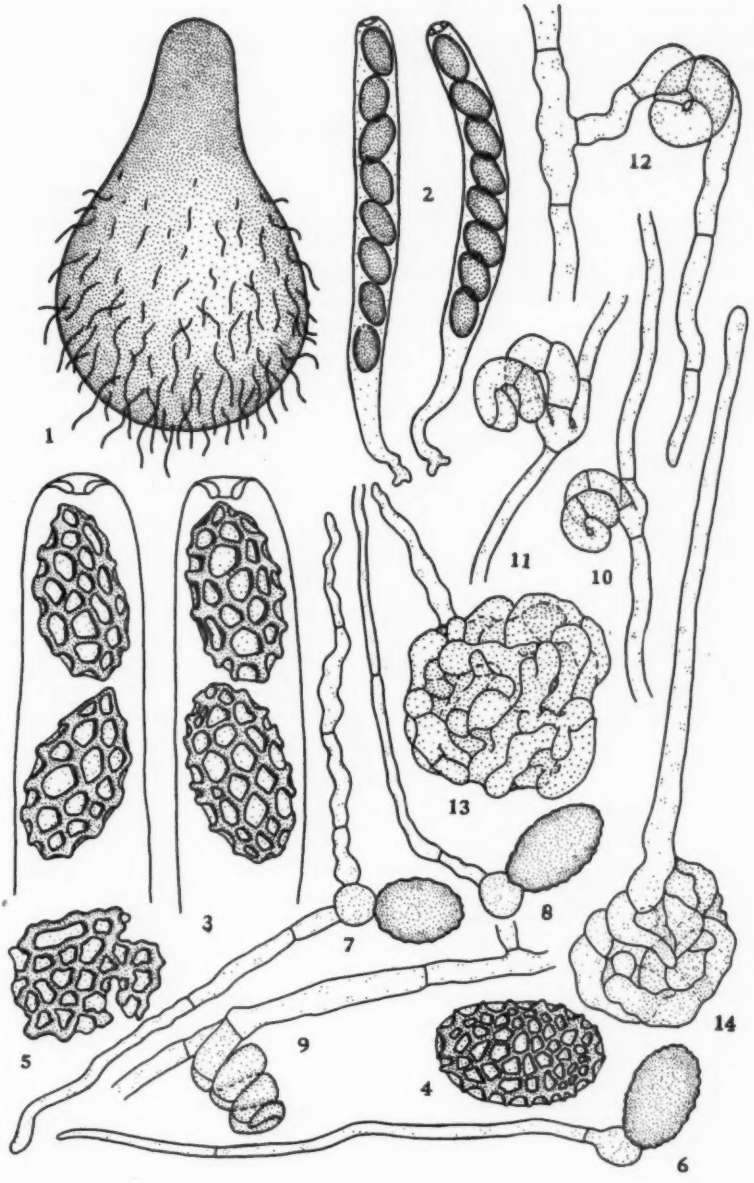
On agar media, producing a rapid development of copious gray mycelium. Self sterile, hermaphroditic. Perithecia developing from coiled ascogonia, superficial or with base slightly immersed, scattered or loosely aggregated, pyriform, 700-1000 × 450-600μ, black, bare except basal part which is covered with light-brownish hyphae; neck papilliform or tapering, smooth, black, bare, 200-400 × 200μ, lined with slender periphyses; wall moderately thick, somewhat coriaceous, nearly opaque, composed of dark brown angular cells measuring 10-20μ. Asci eight-spored, cylindrical, 200-260 × 18-21μ, elongating to over 400μ, broadly rounded to truncate and distinctly perforate at the apex, tapering below into a stipe which becomes considerably elongated at maturity. Large, hyaline, swollen cells surrounding the asci with a few forming filaments up to 12μ in diameter. Ascospores obliquely uniseriate, ellipsoid, somewhat narrowed toward the ends, 22-27 × 12-15μ, dark brown, becoming black and opaque, wall uniformly covered with round or slightly elongated or irregular pits measuring 1.0-1.5μ in diameter; no hyaline sheath surrounding the ascospores. Spermatia ovate, about 2.5 × 2.0μ, budded from short side tubes from short cells of spermatiophores which are upright, aerial, and branching.

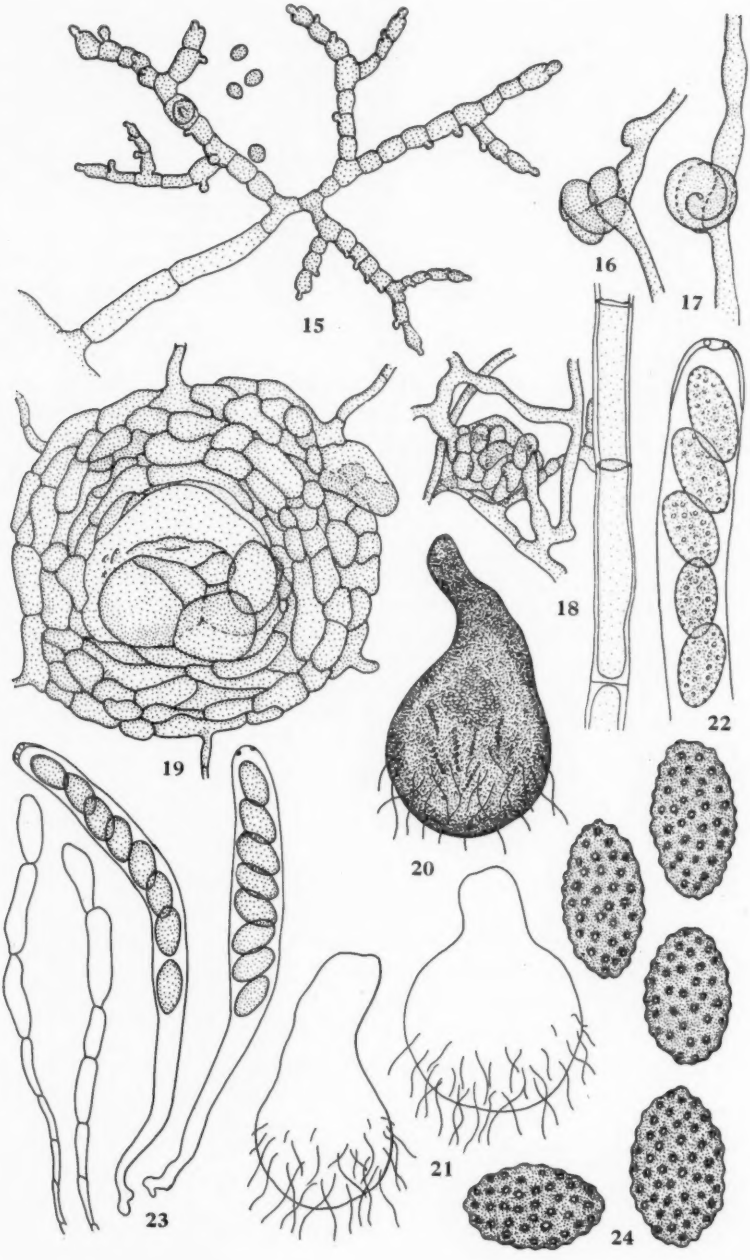
Type: Developed in moist chamber at Toronto on dog dung from Tamsel, Germany, collected by P. Vogel March 1, 1935. Crypt. Herb. Dept. of Botany, University of Toronto *23203* and Herb. RFC *6483*.

Additional material developed in moist chambers at Toronto on cow dung, Tamsel, Germany (RFC *6502*), on horse dung, Duchesnay, Que. (RFC *6950*), and on cow dung, Lac St. Joseph, Que. (RFC *6953*). A specimen of this

---

FIGS. 1-14. *Gelasinospora retispora. Fig. 1. Perithecium.* ×*93. Fig. 2. Two asci.* × *300. Fig. 3. Upper end of two asci with ascospores.* × *900. Fig. 4. Ascospore.* × *900. Fig. 5. Portion of wall from crushed ascospore showing the thickened ridges and large angular pores.* × *900. Figs. 6-8. Germinating ascospores.* × *560. Figs. 9-11. Coiled ascogonia and hyphae.* × *900. Fig. 12. Coiled ascogonium with developing trichogyne filament.* × *900. Figs. 13, 14. Early stages in development of the perithecium.* × *900.*


1
2
3
4
5
6
7
8
9
10
11
12
13
14


15
16
17
18
19
20
21
22
23
24

species was found in the Herbarium of the United States Department of Agriculture, Bureau of Plant Industry, at Washington labelled "*Anthostomella destruens* Shear, (in culture from) *Vaccinium* sp. (blueberry), Wareham, Mass. transfer from culture of Sawyer's, comm. C. L. Shear."

Distribution: Massachusetts, Quebec, and Germany.

Spermatiophores have not been observed on dung substrata. They are sometimes produced in abundance on some media especially those with little or no sugar. On many culture media such as malt agar, no spermatiophores or spermatia are produced and yet perithecia mature in abundance. Under these conditions it is apparent that fertilization is accomplished by means of a fusion between ordinary hyphae and the ascogonium or branches from the ascogonium. The spermatiophores are upright about 100μ in length, aerial, with about four to five main branches each of which is again branched once or twice. The cells are very short, being about 3-6μ long and 4μ wide in contrast with the very long cells in the ordinary hyphae. The cells of the spermatiophore have denser contents. A short side tube is produced from each cell and the spermatia are formed one at a time by budding from the open end, just as in the ordinary phialide. It is probable that the short side tubes represent minute phialides.

The ascogonium develops as a stout side branch with dense contents on an ordinary hypha. It twists into a compact, close coil with ultimately about three turns. As it twists, septa are formed so that four or five cells are produced. Hyphae develop from the basal cell and adjoining filaments and surround the ascogonium. These are at first filamentous and septate but very soon form a pseudoplectenchymatous tissue to produce the wall of the perithecium. The cells in the inner layer remain thin-walled and colorless, those in the outer layer become thick-walled and brown. Each layer is several cells in thickness.

These perithecial initials reach a diameter of 50 to 100μ (occasionally up to 250μ) without fertilization. In monospore cultures the perithecia develop no further. A series of monospore cultures were paired in all possible combinations and it was found that they could be divided into two groups, A and B, such that, when any culture of Group A was crossed with any culture in Group B, mature perithecia with ascospores were formed. Monospore cultures are hermaphroditic but self sterile. However, mature perithecia are frequently developed in cultures of fertile combinations without the formation of spermatia. It is probable that this is accomplished by hyphal fusion. It

---

FIGS. 15-24. *Gelasinospora adjuncta. Fig. 15. Branching spermatiophore showing the short cells with small projections from the tips of which spermatia are budded. Four free spermatia.* × *900. Figs. 16, 17. Coiled ascogonia.* × *900. Fig. 18. Coiled ascogonium being enveloped by hyphae.* × *680. Fig. 19. Young perithecium with unfertilized ascogonium from 12 weeks old monospore culture.* × *900. Fig. 20. Perithecium.* × *46. Fig. 21. Outline of two perithecia.* × *46. Fig. 22. Upper end of ascus showing pore at the apex and five ascospores.* × *560. Fig. 23. Asci and filamentous paraphyses-like structures.* × *300. Fig. 24. Five ascospores showing small pits in the wall.* × *900.*

is likely that a trichogyne filament obtains the required nucleus and transfers it to the ascogonium. But at this stage there are numerous long hyphae produced as branches from the outer cells of the wall and these could not be distinguished from a trichogyne filament.

Anthostomella destruens Shear

This species was described by Shear (3) in 1907 and transferred to *Melanospora* (5) in 1927. The type was isolated in culture from diseased cranberry from New Jersey. This specimen cannot now be found in the Herbarium of the Bureau of Plant Industry in Washington. The author has examined four other collections from this Herbarium. One of these is *Gelasinospora adjuncta* and consists of a dried culture from *Vaccinium* sp. (blueberry), Wareham, Mass., transfer from culture of Sawyer's, comm. C. L. Shear. The other three are *Sordaria fimicola* (Rob.) Ces. & De Not. These are labelled as follows:

(1) Ex *Vitis*. Isolated and det. C. L. Shear. Cultured on *Rubus* stems.

(2) On cranberry, E. Wareham, Mass., No. *329*, dried test tube specimen. May 19, 1928.

(3) *Sordaria uvicola* Viala & Marsais (*Melanospora destruens* Shear – det. C. L. Shear) on *Vitis* sp., type culture from Italy. March 22, 1930.

It is thus apparent that Shear considered specimens of *Sordaria fimicola* as being *Anthostomella destruens*. The photographs published by Shear and Dodge (5) in Plate IF and Plate IIID represent *Sordaria fimicola*. The illustration published by Shear (4) in Plate IV, Figs. 8-11, may refer to *Gelasinospora adjuncta*. This, in all probability, was drawn from the type since in this publication Shear stated that the fungus had been found only once. In the description of the type, the spore size given is nearer *Sordaria fimicola* than *Gelasinospora adjuncta*. There is nothing else in the description that would distinguish between these two species. The gelatinous sheath around the ascospores of *Sordaria fimicola*, and the pits in the wall of the ascospore of *Gelasinospora adjuncta* easily separate the two species, but neither of these characters is mentioned. When mature opaque spores of the latter species are examined in water, it is easy to overlook the pits. The shape of the ascospores is slightly, but distinctly, different in the two species.

If the type of *Melanospora destruens* is found, it may be necessary to reduce *Gelasinospora adjuncta* to synonymy. On the other hand, *M. destruens* may become a synonym of *Sordaria fimicola*.

Sordaria uvicola Viala and Marsais

It was described in 1927 (6) from a culture isolated from diseased grapes. Dried cultures of the type of this species grown on agar, carrot plug, and stems (labelled clover but undoubtedly *Rubus*) located at Washington, have

been examined by the author. These are all *Sordaria fimicola* (Rob.) Ces. & De Not. The perithecia agree in every respect as do the asci which are eight-spored, cylindrical, and with a thickened ring surrounding a pore at the apex. The ascospores are the normal size for *S. fimicola*, but are slightly variable as is usually the case when grown in culture. The lower end is minutely pointed with a round germ pore. They are surrounded by a hyaline gelatinous sheath which, however, does not cover the germ pore.

The description of *S. uvicola* includes, along with the ascomycete, various species of Fungi Imperfecti such as *Alternaria* and a pycnidial form. *S. fimicola* produces coiled ascogonia and the fertilization is not as illustrated by Viala and Marsais. *S. uvicola* was isolated from diseased grapes from Chisinau in Bessarabia. The species is not tenable since it includes a mixture, the ascomycete component of which is *Sordaria fimicola*.

Gelasinospora retispora sp. nov. (Figs. 1-14)

Peritheciis superficialibus, separatis vel gregariis, piriformibus, 700-1000 × 400-600μ (interdum 1200μ longis), nigris, levibus, superne denudatis, pilis longis, flexuosis, hyalinis, septatis, in parte inferiore vestitis; collo conico interdumve longiore, nigro, denudato, 200-300μ longo, periphysibus praedito; membrana perithecii mediocriter crassa, interdum coriacea, opaca, e cellulis atro-brunneis, angulatis, 10-15μ, constituta. Ascis octosporis, cylindraceis, 250-300 × 20-24μ, apice truncatis distincte perforatisque basi in stipitem longum 60-100μ attenuatis. Sine paraphysibus sed cellulis magnis, hyalinis, vesiculiformibus praeditus. Ascosporis oblique monostichis, ellipsoideis, 28-33 × 14-17μ, initio hyalinis dein olivaceo-brunneis usque olivaceo-nigris opacisque, foraminibus angulatis, 2-5μ in diam. et jugis 1-2μ latis praeditis. Spermatiis et conidiis non observatis. Speciebus homothallicis.

Type: Ex seminibus *Beta vulgaris* L. var. *ciclae* L., Enkhuizen, Holland. TRT *23204*.

On agar media producing a rapid growth of white fluffy mycelium. Perithecia developing from coiled ascogonia, superficial, scattered or loosely aggregated, sometimes crowded in small groups on surface of agar, pyriform, 700-1000 × 400-600μ, sometimes reaching 1200μ in length due to more elongated neck, dull black, smooth, bare except the lower part which is covered with white hyphal-like hairs; neck conical, or sometimes more elongate, black, bare, about 200-300μ long, lined with slender periphyses; wall moderately thick, somewhat coriaceous, opaque, with dark-brown, obscure angular cells measuring 10-15μ in diameter; hairs hyaline, flexuous, irregularly and remotely septate, rarely branching or anastomosing, with slightly thickened walls. Asci eight-spored, cylindrical, 250-300 × 20-24μ, truncate at apex with large distinct perforation surrounded by a thickened ring, with a stipe 60-100μ long which gradually tapers to the base, surrounded by hyaline filaments which in the upper part are tubular and in the lower part

with cells swollen to 15μ in diameter or in older perithecia up to 30μ in diameter. Ascospores obliquely uniseriate, ellipsoid, 28-33 × 14-17μ, becoming olivaceous-brown and then olivaceous-black and opaque; spore wall ornamented with low ridges 1-2μ wide which form a reticulate pattern surrounding angular pores which measure 2-5μ in diameter; no gelatinous sheath. No spermatial or conidial stage observed. Homothallic.

The fresh spores germinate in water only after heating (44° C.). They produce a single apical vesicle from which a hyphal filament is produced. On malt agar growth is very rapid and a white fluffy colony completely covering the tube is produced. The hyphae are hyaline or faintly brownish, measuring 3-8μ in diameter. There is considerable aerial growth.

Type: Isolated in culture from seeds of *Beta vulgaris* L. var. *cicla* L. (Swiss chard), Enkhuizen, Holland. Crypt. Herb. Dept. of Botany, University of Toronto *23204*. Additional specimens from same isolation in Herb. Div. of Botany and Plant Pathology, Dept. of Agric. Ottawa, and in Herb. RFC *12105*).

A second collection was isolated from apple twigs, Ste. Anne de la Pocatiere, Que., by A. Payette (No. *595*).

Distribution: Holland and Quebec.

This species differs considerably from the others so far described in the genus *Gelasinospora* in the character of the pits in the ascospore wall. Here they are much larger and angular with the intervening area reduced to narrow ridges. This is considered to be only a specific character and not of sufficient importance for the establishment of a separate genus. Furthermore, there appear to be no other distinguishing features other than the size of perithecia, asci, and ascospores. The admission of this species in the genus *Gelasinospora* requires a slight modification of the original concept.

The ascogonium develops as a side branch from a hyphal thread (Figs. 10-11). It becomes closely coiled and septate with the terminal end extending out as a long trichogyne (Figs. 12-14). Branches from the basal cell (Fig. 11), grow out and enclose the ascogonium to form the perithecial wall (Figs. 13-14).

Acknowledgments

I am deeply indebted to the following for their contributions of material for study: J. Rousseau, J. Savage, the late H. Sydow, P. Vogel, and the late Prof. H. H. Whetzel. Grateful acknowledgment is made to Mr. J. Stevenson for sending specimens from the Herbarium of the U.S. Department of Agriculture, to Dr. J. W. Groves for his contribution of cultures isolated from seeds, to Dr. A. Payette for a culture of *Gelasinospora retispora*, to Mrs. Kaye (née Miss Daphne F.Vick) for inking the drawings, and to Mr. L. Garay for assistance in preparation of the manuscript. I wish to thank Prof. H. S. Jackson for his interest and advice.

References

1. DOWDING, E. S. *Gelasinospora*, a new genus of Pyrenomycetes with pitted spores. Can. J. Research, 9 : 294-305. 1933.
2. PAGE, W. M. A contribution to the life-history of *Sordaria fimicola* (four-spored form) with special reference to the abnormal spores. Trans. Brit. Mycol. Soc. 17 : 296-301. 1933.
3. SHEAR, C. L. New species of fungi. Bull. Torrey Botan. Club, 34 : 305-317. 1907.
4. SHEAR, C. L. Cranberry diseases. U.S. Dept. Agr., Bur. Plant Ind. Bull. 110 : 1-64. 1907.
5. SHEAR, C. L. and DODGE, B. O. Life histories and heterothallism of the red bread-mold fungi of the *Monilia sitophila* group. J. Agr. Research, 34 : 1019-1042. 1927.
6. VIALA, P. and MARSAIS, P. Scléríase des raisins. (*Sordaria uvicola* sp. nov.). Ann. Inst. Nat. Agron. 20 (2E) : 76-135. 1927.

* *In a paper by Claude and Mireille Moreau [Rev. Mycol. 14 (suppl. col. No. 2) : 53. 1949.] a specimen from Congo is described as Gelasinospora calospora (Mouton) Cl. et Mir. Moreau, that is, according to the description, very similar to G. adjuncta. This publication did not come to my attention until the present paper had gone to press.*

(NOTE: *Plates I-IV follow.*)

Plates I-IV

PLATE I. *Gelasinospora adjuncta. Microphotographs of perithecial wall, asci with ascospores, and two separate ascospores showing pits in wall.*

PLATE II. *Gelasinospora adjuncta. Microphotographs of crushed perithecium and ascospores. The mature ascospores appear black and the lighter colored ones with the pits showing are in an immature condition.*

PLATE III. *Gelasinospora retispora. Microphotographs of crushed perithecium, perithecial wall, and ascospores that vary from immature to mature condition.*

PLATE IV. *Gelasinospora retispora. Microphotographs of ascospores varying in maturity from nearly colorless to black and opaque. Note the large angular pores. Near the upper left hand corner is an ascus that is filled with a single ascospore with the characteristic markings on the wall. Some of the ascospores in this area have abnormally elongated pits in the walls.*

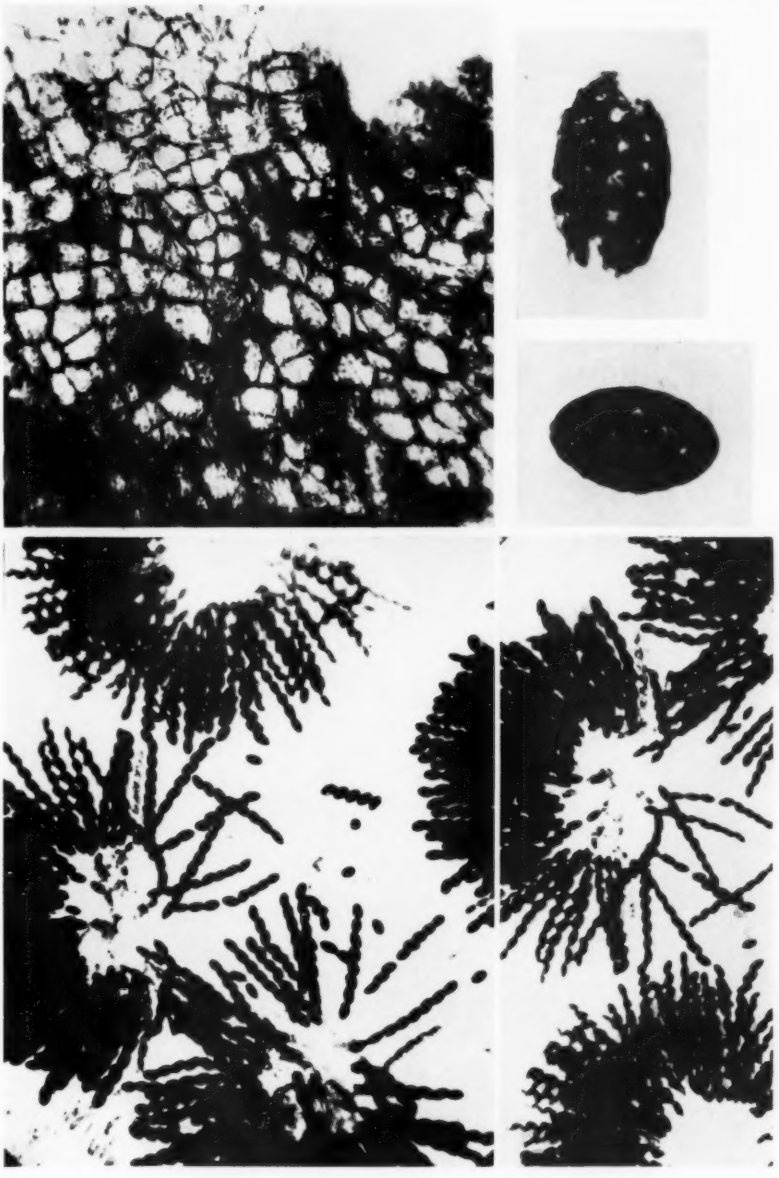
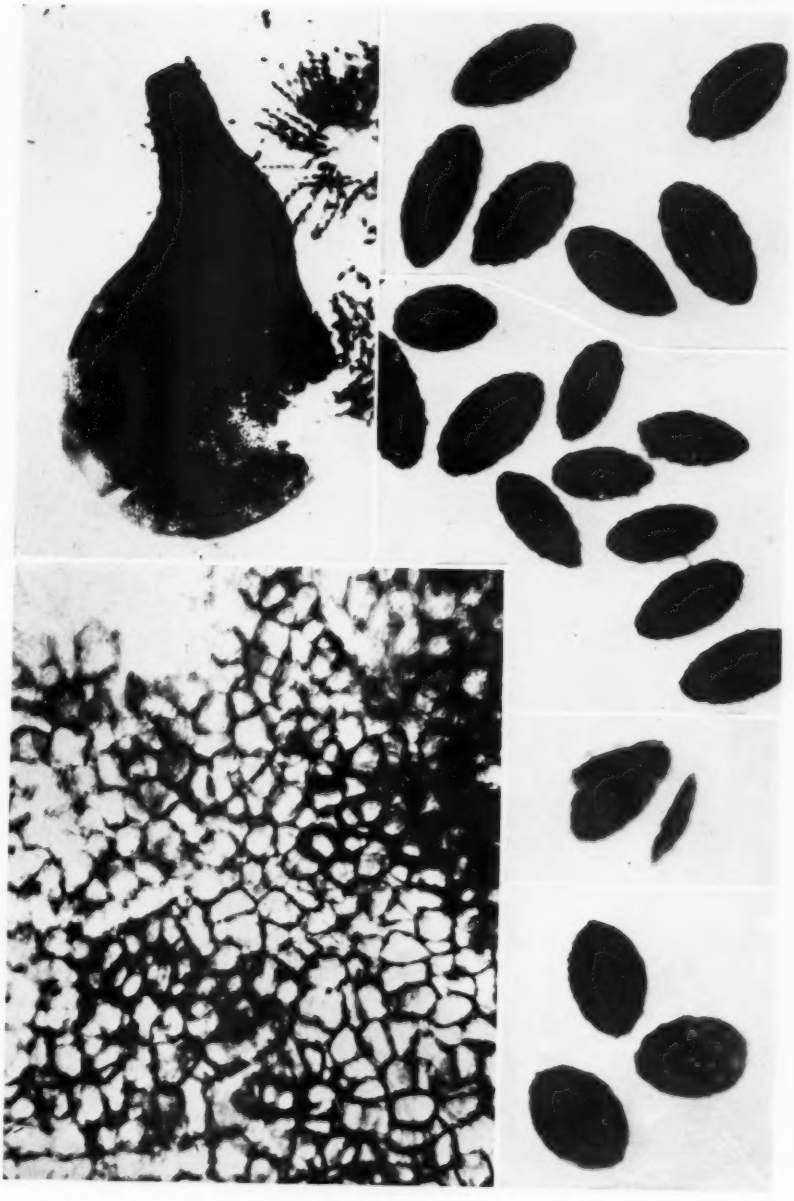


PLATE II

PLATE III



Commonwealth Agricultural Bureaux

This organization was set up by the Government of Canada in collaboration with the other Governments and responsible authorities of the British Commonwealth to provide up-to-date information in the form of journals, technical communications and bibliographies on all aspects of science and practice as applied to agriculture, horticulture and forestry.

JOURNALS:—The following list comprises journals and other periodical publications. Subscription rates are quoted after each; in certain cases (journal marked with an asterisk) a 20 per cent deduction is made for subscribers in the British Commonwealth who send their subscriptions direct.

Bulletin of Entomological Research	(40s.)	*Dairy Science Abstracts	(35s.)
Review of Applied Entomology (Series A)	(40s.)	*Forestry Abstracts	(45s.)
Review of Applied Entomology (Series B)	(20s.)	Forest Products and Utilization	(15s.)
*Review of Applied Mycology	(40s.)	*Horticultural Abstracts	(35s.)
Helminthological Abstracts	(35s.)	*Field Crop Abstracts	(35s.)
*Animal Breeding Abstracts	(35s.)	*Herbage Abstracts	(35s.)
Veterinary Bulletin	(40s.)	*Plant Breeding Abstracts	(35s.)
Index Veterinarius	(100s.)	*Soils and Fertilizers	(35s.)
Nutrition Abstracts and Reviews	(63s.)		

OCCASIONAL PUBLICATIONS

		Price
The Production and Utilization of Silage. A Review of World Literature in Abstracts.	1950.	10s.
Recent Advances in Fruit Juice Production. By V. L. S. Charley and others.	1950.	15s.
The Establishment of Vegetation on Industrial Waste Land. By R. O. Whyte and J. W. B. Sisam.	1950.	10s.
Studies on Micro-Fungi, 1. The Genus *Fusariello* Saccardo. By S. J. Hughes.	1949.	2s. 6d.
Wilt of *Calophyllum inophullum* L. var. *Tacamaha* (Willd). By O. O. Wiehe.	1949.	3s.
Terminology in *Phytophthora*. By Elizabeth Blackwell.	1949.	4s. 6d.
List of Recorded Cotton Insects of the World.	1948.	5s.
West African Meliolineae. II. Meliolineae Collected by F. C. Deighton.	1948.	12s.
A List of Plant Diseases of Economic Importance in Tanganyika Territory.	1949.	3s. 9d.
List of Common Tropical Plant Diseases.	1947.	3s.
Seed production of European Vegetables in the Tropics.	1948.	2s.
Growth Substances and Their Practical Importantce in Horticulture.	1948.	12s. 6d.
Five hundred Varieties of Herbage and Fodder Plants.	1948.	15s.
The Technique of Breeding for Drought Resistance in Crops.	1948.	2s. 6d.
New and Promising Varieties Recently Described in the Literature.	1948.	2s. 6d.
The Practice of Soil Conservation in the British Colonial Empire.	1949.	10s.
Bibliography of Soil Science, Fertilizers and General Agronomy.	1944–1947.	35s.
New Techniques for Mounting Fungi.	1949.	1s. 6d.
Proceedings of the First Commonwealth Conference on Tropical and Subtropical Soils.	1948.	25s.

All correspondence regarding above journals and other publications may be addressed in the first instance to:—

COMMONWEALTH AGRICULTURAL BUREAUX,
CENTRAL SALES BRANCH,
PENGLAIS,
ABERYSTWYTH, GREAT BRITAIN.

CANADIAN JOURNAL OF RESEARCH

Notes on the Preparation of Copy

GENERAL:—Manuscripts should be typewritten, double spaced, and the **original and one extra copy** submitted. Style, arrangement, spelling, and abbreviations should conform to the usage of this Journal. Names of all simple compounds, rather than their formulae, should be used in the text. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed. Manuscripts should be carefully checked before being submitted, to reduce the need for changes after the type has been set. If authors require changes to be made after the type is set, they will be charged for changes that are considered to be excessive. **All pages, whether text, figures, or tables, should be numbered.**

ABSTRACT:—**An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required.**

ILLUSTRATIONS:

(i) *Line Drawings:*—All lines should be of sufficient thickness to reproduce well. Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper **ruled in blue only**; any co-ordinate lines that are to appear in the reproduction should be ruled in black ink. Paper ruled in **green, yellow, or red should not be used** unless it is desired to have all the co-ordinate lines show. Lettering and numerals should be neatly done in India ink preferably with a stencil (**do not use typewriting**) and be of such size that they will be legible and not less than one millimetre in height when reproduced in a cut three inches wide. All experimental points should be carefully drawn with instruments. Illustrations need not be more than two or three times the size of the desired reproduction, but the ratio of height to width should conform with that of the type page. **The original drawings and one set of small but clear photographic copies are to be submitted.**

(ii) *Photographs:*—Prints should be made on glossy paper, with strong contrasts; they should be trimmed to remove all extraneous material so that essential features only are shown. Photographs should be submitted **in duplicate**; if they are to be reproduced in groups, one set should be so arranged and mounted on cardboard with rubber cement; the duplicate set should be unmounted.

(iii) *General:*—**The author's name, title of paper, and figure number should be written in the lower left hand corner (outside the illustration proper) of the sheets on which the illustrations appear.** Captions should not be written on the illustrations, but typed on a separate page of the manuscript. All figures (including each figure of the plates) should be numbered consecutively from 1 up (arabic numerals). **Each figure should be referred to in the text.** If authors desire to alter a cut, they will be charged for the new cut.

TABLES:—Titles should be given for all tables, which should be numbered in Roman numerals. Column heads should be brief and textual matter in tables confined to a minimum. **Each table should be referred to in the text.**

REFERENCES:—**These should be listed alphabetically by authors' names, numbered in that order, and placed at the end of the paper.** The form of literature citation should be that used in the respective sections of this Journal. **Titles of papers should not be given in references listed in Sections A, B, E, and F, but must be given in references listed in Sections C and D.** The first page only of the references cited in papers appearing in Sections A, B, and E should be given. **All citations should be checked with the original articles.** Each citation should be referred to in the text by means of the key number; in Sections C and D the author's name and the date of publication may be included with the key number if desired.

Reprints

Fifty reprints of each paper without covers are supplied free. Additional reprints, if required, will be supplied according to a prescribed schedule of charges. On request, covers can be furnished at cost.